

Mrs Thatcher and the Universities

THOSE who may have hoped that the months ahead would see a better relationship, or at least a more logical relationship, between the British government and British educational institutions will have been disappointed by the exchange last week in the House of Commons between Mrs Margaret Thatcher, Secretary of State for Education and Science, and the robust company of her critics and lukewarm friends at the end of the debate on the Queen's Speech which outlined the British government's programme of legislation for the year ahead. The first thing to be said, of course, is that there is now very little chance of any but comparatively minor administrative changes in the way in which young men and women in Britain are educated. The school-leaving age will be raised to 16, but that has been on the cards for several years. The Queen's Speech (written by the Prime Minister and his colleagues) says that "provision for higher and further education will be improved and expanded", but there is very little in the record of the debate on November 5 which suggests that those words are more than platitudes. To be sure, Mrs Thatcher roused to anger some of her most fierce critics by announcing the decision that in future students' unions would have to look for support to their universities and not to the local authorities accustomed to pay fees, but that is hardly a matter on which critics have temperate things to say. And the sad truth is that Mrs Thatcher herself contributed nothing to resolving the doubt which still surrounds the future of the British university system and its relationship with the other instruments of higher education.

Why should this be? Is the British government entirely powerless in the management of its own affairs? For the past six years, the previous Labour government was occupied in making water flow uphill by advocating the binary system of higher education, a mixture of universities and polytechnics. At this stage, nobody can know whether the intention was to discover a cheaper way to higher education than that of the traditional universities, a more egalitarian formula for the admission of students or genuine diversity. The fact is that by setting out to create polytechnics, the British government appeared to wash its hands of the universities. But it is clear that the polytechnics, dependent as they are on local rates and taxes, are also outside the government's ken. Paradoxically, it was the Labour government that found what now appears to be a formula for shrugging off the obligation of central government for higher education. In spite of the painful anomalies which suffuse the relationships between polytechnics and universities, it is perhaps only natural that the present government should put up with them for the sake of a quiet life. The trouble, of course, is that both parts of the binary system at present exist in a kind of limbo, not knowing what will happen next. On last Friday's form, neither partner in the binary system would be advised to look to Mrs Thatcher for guidance. She had literally nothing to say on this important issue.

Her best defence is that most of her potential critics seem to have been so bemused by issues such as the proposal to strengthen the financial position of direct grant schools and to undermine the automatic autonomy of students' unions that they had nothing to say about the central issue of how best to organize higher education.

The sad truth is that there is no reason why Mrs Thatcher should be at a loss to know what now to do. First of all, of course, there is a certain arithmetical inevitability about the pattern of higher education. Each year, more students find their way into universities and polytechnics. Although governments are inclined to take the credit for the pace of this expansion, it happens to be a kind of historical necessity that the university population should grow at such a rate as to double in just over a decade. Eventually, of course, when most of the population finds its way into higher education, the pace of growth will slacken, but for the time being any British government is bound to a kind of chariot wheel that will not stop turning until the 1980s are over. To claim credit for this is like boasting at having arranged sunrise at some previously advertised time of day.

What Mrs Thatcher really needs to tackle is the question of how to pay for higher education and, in the same spirit, how to organize it. First of all, it is anomalous and debilitating that universities and polytechnics should be organized quite differently, with central government funds for one and local authority funds for the other. Why not amalgamate the two elements in the binary system? Second, it is absurd that instruments such as the University Grants Committee should be dedicated to the rule that all institutions of the same category must be counted equal. This spurious egalitarian spirit was a serious source of discontent about the university system when there were only universities to worry about. It would have been better for the health of university education if it had been easier for universities with ambitions in, say, classics or photography to pursue their particular interests without the fear that they might fall foul of the University Grants Committee and its improving standards. To be sure, such independence from preconceived notions of what a university should be is only possible when universities themselves raise at least a part of the money they have to spend. The moral for Mrs Thatcher is that the health of higher education as well as the administrative convenience of her administration require that there should be not so much a binary system of institutions but a binary system of sources of finance. In practice, this requires higher university fees, paid for by local authorities, more independence for polytechnics, more responsibility by individual institutions for determining what constitutes a decent course in higher education and a greater sense among students that the whole thing is worth having, whoever pays the subvention for the students' union. Why is it that Mrs Thatcher seems entirely to have shrunk from that simple declarative position?



The Importance of William M. Magruder

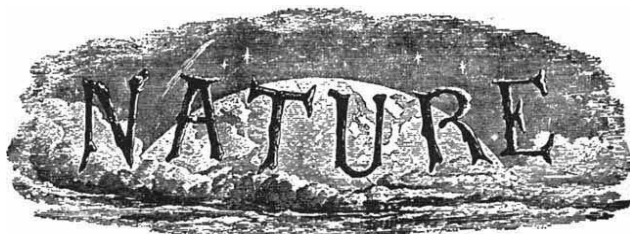
LESS than a year ago, even Mr William M. Magruder's fondest admirers, of whom there are many, must have been downcast at the collapse of his campaign to persuade Congress to sponsor the supersonic transport aircraft project. Even at the time, it seemed as if a more subtle person might have been more successful. Mr Magruder has a reputation as a salesman of new ideas, but he is a high pressure salesman—the kind of man who tends to begin his sales pitch by jamming his foot in the door. It is no wonder that Congress's unwillingness to stomach the SST was if anything strengthened by the sense which Mr Magruder gave a great many senators that there were no options open to them. In retrospect, it is of course extremely strange that such an experienced operator as Mr Magruder should have failed to anticipate the consequences of a refusal by Congress to vote the money for which he asked. Yet, to the Administration's chagrin, there appear to have been no contingency plans. Lesser men than Mr Magruder would no doubt have quickly found themselves banished to the outermost regions of the United States. It is therefore something of a surprise that Mr Magruder has now emerged as the head of what is called the New Technological Opportunities Program, a still somewhat amorphous scheme for winning public benefits of various kinds from the opportunities which technology now provides.

Quite apart from the question whether Mr Magruder is the right man for such an intricate job, the decision to set up a White House organization for the exploitation of technology raises all kinds of difficult problems, both in the management of the United States government's interests in science and technology and in the process of forming an accurate appreciation of what science and technology may do for a society such as that of the United States. The most obvious line of speculation since Mr Magruder's new job became known is the extent to which disappointment will cut across the operations of the Office of Science and Technology, hitherto the principal channel for the coordination of the federal government's activities in the support of basic science and in some fields of technology as well. From Dr Edward David down, the air has been thick with loyal protestations that the new arrangements imply no diminution of authority for the Office of Science and Technology and some have even said that there will be benefits in having Mr Magruder at the White House to lend support to projects which seem to have a technological flavour of some kind. Unfortunately, however, the situation is a good deal more complicated than that. In the past few years, the Office of Science and Technology has done a great deal to strengthen the representation of agencies such as the National Science Foundation in the annual competition for budget appropriations.

The biggest danger now is that Mr Magruder, busy as a bee as is his habit, will muddy the waters unnecessarily. Even in the United States, there are serious limitations to the uses which can be made of technology. People say, quite rightly, that the threat of another earthquake in San Francisco is sombre, that the prospect of hurricanes each autumn is an affront against human dignity and that it should somehow be possible to get rid of the constant embarrassment about the balance of payments by wringing a little more blood out of the stone of technology. The

trouble, alas, is that there is no easy way of knowing precisely when it is possible by means of a strictly technological development to make wishes into realities. The objection to much of what has in the past few years been said of the power of technology to cure social ills of various kinds is that the wish has been the father of the thought. The truth is, of course, that even when it is acknowledged that cancer, for example, would be best got rid of, or when society as a whole yearns for the removal of earthquakes, there is a limit to the amount of money which can be usefully spent in the attainment of these desirable objectives. The danger now is that Mr Magruder's brash new broom will be sweeping away energetically for a good time before this simple lesson has been re-learned. The result will be, if the worst fears are fulfilled, that the cause of rationality in the applications of technology to practical problems will have been set back by several years. Will Mr Magruder be more perceptive in his handling of this question than in his dealing with the supersonic transport?

100 Years Ago



But the most interesting, perhaps, of all the subjects with which the *Académie des Sciences* busied itself, was that of seeking an economical means of alimination for the inhabitants of Paris during the siege. Given certain limited sources of supply, a fixed amount of suitable organic matter, and the problem was how to use the same to the fullest and most profitable degree. Of sheep and oxen there was but an exceedingly limited provision in proportion to the very populous state of the city, and although corn and wine were said to be in abundance, there is no doubt the authorities were from the first sorely troubled by the vague estimates that were published of these comestibles.

A proposition to manufacture artificial milk, brought forward by M. Gaudin, seems worthy of some notice. That gentleman estimated that 500,000 litres per day of milk could be prepared in Paris at an exceedingly trifling cost, which should have all the nutritious qualities of good milk, and which should, besides, be neither unpleasant of taste or smell. An emulsion at a very high temperature is made of *bouillon de viande* prepared from bones, fat, and gelatine, and when cold, a product is obtained resembling in taste stale milk of a cheesy flavour; the components of ordinary milk are all present, the gelatine representing the casein; fat, the butter; and sugar, the sugar of milk. For admixture with coffee, chocolate, soup, &c., the milk is said to be by no means disagreeable.

Many propositions were brought forward to economise the blood from the abattoir, the plan suggested by M. Gaultier of mixing it with flour in the manufacture of bread being perhaps the best and simplest, as the fibrine and albumen, so rich in nitrogen—of which the alimentary properties are well known—are in this way utilised to the highest degree. Less inviting is the proposal of M. Fud to consume the carcasses of animals that died of typhus, rhinderpest, and other diseases, the flesh in these instances being, so asserts M. Fud, capable of use as food, if only cooked in a suitable manner.

From Nature, 5, 45, November 16, 1871

OLD WORLD

TELEPHONES

Anachronism on the Way

from a Correspondent

THE British Post Office has surprised nobody and disappointed many by finally deciding on a large analogue switching system for its telephone exchanges in the late 1970s and early 1980s. The system, called the TXE-4, has been under development by Standard Telephones and Cables Ltd, one of the Post Office's traditional equipment suppliers. But as the TXE-4 handles analogue signals only, it cannot mesh with the pulse code modulation digital transmission systems which the Post Office is installing and boasting about. As there is no doubt that the telephone system will eventually be all-digital—as it must be to handle computer data, television and voice signals indiscriminately at high speeds—the TXE-4 is, before it is installed, an anachronism. To adopt it for the future is like adopting a design of traffic roundabout that cannot accept traffic from the latest motorways.

But the Post Office, like the heart, has its reasons. The chief advantage of the TXE-4 is that it will speed the replacement of the old-fashioned Strowger switching on which 95 per cent of the national telephone system still depends. The TXE-4, based on reed-relays, is a semi-electronic exchange. It can provide up to 70,000 lines—the size of a large local telephone exchange—and resembles the TXE-2, the smaller semi-electronic exchange which the Post Office is already ordering at the rate of three a week. This type of switching is the only system available that is truly compatible with the existing Strowger. Moreover, it is ready, or almost ready—STC has one developmental model working (although at costs that are nowhere near competitive with Strowger exchanges). The £15 million contract that the Post Office has given STC will allow the company to put the TXE-4 into production and to give the Post Office, under this contract, eighteen of them.

Mr Edward Fennessy, managing director of Post Office telecommunications, has conceded that in making this major and long-deliberated decision, the Post Office may be accused of over-conservatism. So it might. There had been hopes that the Post Office would decide on a computer-controlled switching system, preferably an all-digital one. But to do so would have meant a delay of several years. The enthusiasts for the TXE-4 say that it can be computer-controlled when the need arises and that it will therefore not be obsolete—merely obsolescent

when the all-digital communications network arrives. The Post Office clearly has bent over backwards to avoid repeating the disastrous mistake of the 1950s—the decision to go for all-electronic switching without an intermediate stage. It is being super-cautious, and perhaps the chance for practical immediate improvements to the existing telephone network will justify it.

But exchange equipment is big business, one which gave British telecommunications manufacturers the leading position in world markets. Nobody abroad is going to buy the TXE-4, it seems safe to say. It is too eccentrically British to have much appeal abroad. (The expense of its development has been rebuked by the Committee on Public Accounts.) Neither will it achieve another objective supposedly held by the Post Office—to stimulate competition. If in December 1972 the Post Office decides to install the TXE-4 on a major scale, STC will probably teach GEC-AEI and Plessey how to make it. Thus the old circle of suppliers—the ring—will be just as closed to outsiders as it was before Parliament asked the Post Office to act as a real business in the outside world. The alternative would have been for the Post Office to buy large semi-electronic exchanges abroad and let the British industry get on with the development of the more advanced computer control exchanges which the Post Office will need very soon and which have great export potential.

RESEARCH AND DEVELOPMENT

Small Firms Seedbed

THE theme of the Bolton report published last week is that the British Government should remove the inequalities and disabilities from which small firms now suffer (*Report of the Committee of Inquiry on Small Firms, HMSO, £2.55*). "Benign neglect" is how Mr J. E. Bolton described the government's attitude to small firms so far, and the government's immediate response has been the establishment of a small firms division within the Department of Trade and Industry, as the Bolton report requested.

Deep in the 436 pages of the report are its findings on research and development in small companies (broadly speaking, companies with less than 200 employees), based on one of the eighteen research reports commissioned by the committee (see *Nature*, 233, 226; 1971), but the committee has considered other sources and arrives at some conclusions.

The committee scorns the suggestion that new ideas are excessively costly for small companies to pursue, pointing out that small companies "continue to make

major inventions . . . even in the areas of the most advanced technology". The committee goes on to say, however, that "they cannot possibly play a major innovative, as distinct from inventive, role in activities requiring very large numbers of employees and capital such as airframe manufacture". While warning that the statistics available must be interpreted with caution, the report concludes that, on the question of invention as distinct from innovation, small companies "make a disproportionately large contribution, particularly in relation to their expenditure on research and development". The increased costs of research and development are not seen to be a contributory factor to the overall decline of the small company which the committee notes, as costs are in general highest in industries which are already concentrated—for example the aerospace and chemical industries. The greater availability of research facilities from industrial research establishments, government establishments and universities is making research easier for small companies and the report says that there is ample evidence that economic growth is impaired more by a failure to apply existing knowledge than by shortcomings in new research and development.

None of the report's sixty recommendations relates specifically to research and development in small companies, but the general tenor of the report is to make life easier for them and this can only encourage their inventive work. That the Bolton committee considers this aspect of their work to be important is shown by the last three of the eight functions of small firms that the report distinguishes, namely

- that small companies, although they account for a small proportion of expenditure on research and development, are an important and low-cost source of innovation in products, techniques and services,
- that small companies are a breeding ground for new industries—that is for innovation writ large,
- that small companies are the seedbed from which new large companies will grow.

In a statement on the report, Mr Bolton, the committee's chairman, said that "this seedbed function appears irreplaceable" and that the most important question which faced the committee was whether small companies will "survive and flourish in sufficient numbers to continue to provide the seedbed of new enterprise".

NUCLEAR POWER

Which Way to Turn?

A REASONED plea is made in the latest issue of *State Service*, the journal of the Institution of Professional Civil Ser-

vants, for the British government to adopt the Steam Generating Heavy Water Reactor (SGHWR) as the next step in the British nuclear power programme. Mr John Lyons, deputy general secretary of the IPCS, says that electricity generation would be cheaper and export potential higher if the SGHWR were adopted instead of the Advanced Gas Cooled Reactor (AGR) which the British government is at present supporting.

The Department of Trade and Industry is studying the next step in an official committee. The question is whether to continue with the AGR or to adopt the SGHWR. The committee is also considering whether the High Temperature Reactor (HTR) or the American light water reactor could be used as the basis of British nuclear power generation until the fast reactor, now under development, is phased in. Mr Lyons dismisses the possibility of using the American light water reactor as, in his view, the British reactors are better than their American counterparts.

The HTR on the other hand is the reactor actively under consideration in Britain as the next step after the AGR. Its development is, however, seven or eight years behind that of the SGHWR, now ready to go into production. The decision therefore boils down to whether to continue building AGRs until the HTR becomes a practical possibility—and there is no guarantee of this—or to abandon the AGR and base the British nuclear programme on the SGHWR for the next ten years. Mr Lyons emphasizes that a decision to adopt the SGHWR would not mean relinquishing the HTR and he says that development work should continue.

The reasons for replacing the AGR by the SGHWR are, according to Mr Lyons, three-fold. First, the capital cost and cost of electricity generation by the SGHWR will be substantially less than that of the AGR. Second, the SGHWR is better able to follow the demand for electricity and simpler to construct and maintain. It also produces more plutonium—an important factor for the fast reactor programme. Third, the SGHWR has a high export potential whereas the AGR has none.

Mr Lyons points out that the decisive factor which should make the committee recommend the SGHWR is its export potential. It remains a sore point that Britain has only exported two nuclear power reactors—both of them of the Magnox type. Mr Lyons argues that once a country has invested in a water reactor, it is more likely to continue with a similar type. Thus the SGHWR is more likely to be sold abroad than the AGR which Britain has completely failed to export so far.

Mr Lyons also demonstrates his lack of faith in the British ability to maintain

its two-year lead in fast reactor development by saying that when the time comes for Britain to export its fast reactors, the Americans and Europeans will have caught up, and unless Britain has a footing in the export market by then it will be severely handicapped.

The Central Electricity Generating Board has so far snubbed the SGHWR although, according to Mr Lyons, the chairman, Sir Stanley Brown, has acknowledged its suitability. The CEBB has opted for the HTR to follow the AGR because it is more likely to have an edge over the SGHWR in the larger reactors that the CEBB will need. Also part of the argument for continuing with the AGR for the next few years is that the CEBB staff are trained in gas reactors and that the introduction of a water reactor would mean acquiring a new technology.

HOVERCRAFT

Lift-off

from a Correspondent

STATIC tests are now under way on Britain's hovertrain after four years of development by Tracked Hovercraft Ltd. Running tests will be made on the mile long track at Earith near Cambridge in a few weeks' time.

Two British developments form the basis of the hovertrain. The jet air cushions supporting the hovercraft were pioneered by Sir Christopher Cockerell and the propulsion system is a single sited linear induction motor invented by Professor Eric Laithwaite of Imperial College, London. The combination of hoverpads and linear motor will allow operational speeds of up to 300 mph. The propulsion system itself is quiet and the noise from the air pad fans is not excessive, although at present the RTV 31 test vehicle is fitted with unsilenced fans so that noise sources can be easily located. A progressive noise reduction programme is aiming at 90 db—comparable to a heavy lorry—as an acceptable noise level. The test vehicle is remotely controlled with the equipment on board telemetered by the control centre. A second experimental craft is nearing completion and the two vehicles will be used for future development of the suspension, propulsion and guidance systems.

A further grant of £1.75 million in addition to the £3.6 million already loaned to THL was confirmed by the British Government in September and this will allow the extension of the elevated track from three to eight miles so that speeds of 250 mph should be reached by the end of 1972.

Interest in the hovertrain is rapidly increasing. Within the past month the United States Department of Transportation has officially endorsed the

British system for the congested north-east corridor—the Washington–New York–Boston run. Tracked Hovercraft Ltd is convinced that the key factor in the cost of hovertrain systems, which are also being developed in France and the USA, is the cost of the track. The American system uses a U-shaped trough whereas the British system uses a simple box beam and cost analysis of the two systems showed the THL system to be cheaper by 20 per cent—a saving of \$700 million over the projected route. The department's report recommended that planning of the route should begin immediately and that the decision on which system to use should be made in 1976.

THL has also studied the possibilities of hovertrain links between London and Foulness, Gatwick and Heathrow, and between London and Liverpool. It estimates the cost over a 200 mile run, including stations, vehicles, construction and land acquisition, to be appreciably under £1 million per mile, the cost of motorways. A commercial application of the hovertrain would operate on pylons spaced up to 150 foot apart and 25 to 30 foot off the ground which eases the cost of land acquisition and causes less disruption than motorways. THL also estimates that the service could be run at a 10 per cent return on capital invested at rates between British Rail's first and second class fares.

THL claims, and the rest of the world appears to be beginning to agree, that its system is faster, quieter, cheaper and simpler than its French and American rivals and it is confident that, provided no unexpected snags appear during testing, the hovertrain is both a practicable and an economic proposition for long distance links.

Appointment

PROFESSOR S. F. EDWARDS, University of Manchester, has been appointed by Mrs Margaret Thatcher, Secretary of State for Education and Science, to fill one of two outstanding vacancies on the University Grants Committee. His term of office will run until 1976. The other members are Sir Kenneth Berrill, chairman, Sir Robert Aitken, Professor G. A. Barnard, Professor C. E. H. Bawn, Professor J. Black, Dr G. S. Bosworth, Miss E. J. Bradbury, Professor A. J. Brown, Dr D. Cook, Professor R. C. Cross, Professor J. Cruickshank, Professor A. Davies, Professor J. Diamond, Mrs J. Floud, H. R. Galleymore, Professor N. C. Hunt, Professor D. Lewis, Professor P. G. Stein, and Professor Sir Charles Stuart-Harris.

Experimenters Incommunicado



THE characteristics of the Post Office Tower in London under stress have, as is well known, been tested in a recent experiment, the authorship of which is at present unknown even to the London police (see picture). It is less well known that a more carefully controlled study of the building has been carried out by the staff of the Building Research Station and recently published (*Strain Measurements at the GPO Tower, London*, K. J. Eaton and J. R. Mayne, CP29/71). The lack of communication between the two groups of experimenters will no doubt be regarded as a further sign of interdisciplinary isolation.

STANDARDS

All Things being Equal

THE International System of units (SI) was rounded off for the time being by the decisions of the 14th General Conference of Weights and Measures (CGPM) in Paris last month. The conference agreed that the mole should become a base unit of substance within

the SI with the pascal and siemens being approved as SI units of pressure and conductance, a pascal being equal to a newton per square metre and a siemens equal to a reciprocal one ohm. The CGPM's forty member states meet at least once every six years to make decisions about units, but its function is really to approve the work of the International Committee on Weights and Measures which meets at least once every two years. Once approved, SI units are published by various bodies throughout the world and by the British Standards Institute (BSI) and HMSO in Britain.

They are also published by the International Organization for Standardization, the international authority on names and symbols for physical quantities and the relation between quantities and units. Seven parts of ISO recommendation R 31 have so far been published on quantities and units, with three parts passed for publication and four more in draft.

Before publishing a recommendation, ISO consults national standards organizations and other interested bodies such as the International Union of Pure and Applied Physics, the International Union of Pure and Applied Chemistry and the International Electrotechnology Commission, all of which have their own committees working on standardization of language, symbols and units, and ISO often takes over their agreements. By the time a standard is published it will have been commented on at least two or three times by the interested bodies of the forty or so countries that participate in ISO. As a result, about ten years elapse from the beginnings of agreement on a standard to publication. But the proposed new standards are published and tend to be used before they are officially approved—as has been the case with the SI pascal and siemens.

The future of standards lies simply in efforts to reach greater agreement and in deciding on units and symbols for new fields of science, although, according to Professor McGlashan of the University of Exeter and IUPAC, "more progress has been made in the past twenty years than in the previous ten thousand".

The next meeting of the CGPM in 1975 will celebrate the centenary of the signing of the Metre Convention and it is unlikely that there will be any change made in the base units of SI. The basic work seems over for the moment, but work will no doubt continue in the redefinition of standards—for example the 10th conference in 1960 redefined the metre as "the length equal to 1,650,763.73 wavelengths in vacuum of the radiation corresponding to the transition between the levels $2p_{10}$ and $5d_5$ of the krypton-86 atom," replacing the definition of 1889 based on the platinum-iridium bar.

WORLD WILDLIFE

Only Skin-deep

IN the presence of such personalities as Sir Charles Clore, Lady Gage, Miss Iris Murdoch, the editors of *Vogue* (both British and American versions), Mrs Rolf Harris and two buyers from Harrods Ltd, Aquascutum, in collaboration with the World Wildlife Fund, revealed its collection of simulated fur fashions earlier this week.

On display, suitably draped around six of the potential Miss Worlds, to the accompaniment of recorded jungle drums and lashings of champagne, were coats made from simulated cheetah, leopard, ocelot and tiger in "styles created especially to celebrate the 10th Anniversary of the World Wildlife Fund". A number of puma and tiger cubs imported for the occasion—presumably to show what the real thing looks like—started off by gambolling around and ended rather frightened at the pressing enthusiasm of their well-wishers.

Mr Peter Scott, chairman of the British National Appeal, opened the proceedings by saying that he hoped the wearing of real fur will die out as a fashion (cheers), that the Ministry of Defence has agreed not to buy real fur for military drummers to wear (patriotic cheers) and that the sale of the synthetic furs would help conserve those predatory species in danger from man (yet more cheers).

The coats, from man-made fibres cost less than £40 compared with £1,200 to £2,000 for a real ocelot coat. How much do the simulated coats cost to make? Well, Aquascutum for one isn't saying, but judging by the number of women surreptitiously trying them on at the end, they are going to be quite the thing to be seen in. Were they as good as the real thing? Well, no, of course not," said one eager purchaser, slipping out of a (simulated) leopard into a (simulated) cheetah, "but one's friends won't talk to one if you wear real fur; I mean when there are only 200 left in the world you're not going to be very popular if you appear wearing five of them, are you?"

Meanwhile, back on the dais, Miss World 1969—Miss Eva Reuber-Staier—wearing a (simulated) jaguar coat was playing with the young cats to the delight of the adoring multitudes and the frantic photographers—"a little more leg, Miss Reuber-Staier?". Cameras flashed, people cooed, and Peter Scott signed autographs in a corner—simulated fashion furs had definitely arrived.

And if the cats seemed less delighted than the models at the bright lights and brighter people, can you really blame them?

Success and Failure

THE past few weeks have seen some ironical developments for European and British space research. Britain, which no longer wants a national launch vehicle, now has one, and ELDO is still without the launcher which is the chief purpose for its existence. The avowed British policy of depending on the United States for future launch facilities, both for scientific and technological satellites, has, however, now been vindicated (at least partially). At last, the anomaly of continuing with this inadequate British launcher, contributing to ELDO and planning to make increasing use of American facilities has been resolved, and the situation is now less confused than at any time since Britain joined ELDO while at the same time developing Black Arrow.

Black Arrow's successful launch, on October 28, and the failure of Europa II on November 5 lend weight to the British decision to stop financial support for ELDO. Coming only a few days after the announcement that the US State Department has now agreed in principle to provide launch facilities for research, meteorology, navigation, telecommunication and Earth resources satellites, the most recent failure may well sound the death knell for ELDO. The only members of the organization still enthusiastic about an independent European launcher are France and Germany, who may proceed with a bi-national programme aimed at providing a launcher for Symphonie.

Just what did go wrong with Europa II? It is too soon to be sure, but indications are that the failure—possibly an explosion—occurred 150 s after take-off in the first stage, Britain's Blue Streak. This is the only stage which has never failed on previous ELDO launches. But the rocket's navigation system also failed, before the explosion and, apparently, independently of it. A second explosion, in the French Coralie second stage, may have been triggered by the Blue Streak failure. It is a sobering thought that roughly 100 proved American launchers could have been bought for the £250 million spent by ELDO so far.

The future for European satellites, however, looks brighter than the gloomy launcher position might indicate. For although it has been clear for years that because the cost per launch decreases as a vehicle is used more, American launchers are and will remain cheaper than European launchers can hope to be. Until last week there was no firm promise that such launchers would be available except for scientific satellites or for projects with a large American interest, such as Intelsat. There are,

however, no apparent strings attached to the new promise, although it may well be intended to create an atmosphere of cooperative goodwill towards the shuttle project.

BRUCELLOSIS

Towards Clean Cows

IN its campaign to wipe out brucellosis, an infectious disease that causes spontaneous abortion in cows and undulant fever in people, the Ministry of Agriculture last week restricted cattle movements in certain areas of Great Britain.

Although vaccination for calves has been available since the 1940s, the first real steps were taken in April 1967 with the introduction of the Brucellosis (Accredited Herds) Scheme which built up a register of herds free from brucellosis, chiefly to be used as replacement cattle for later stages of eradication. In July 1970, the Brucellosis Incentives Scheme was introduced, which provides financial inducements of £0.008 per gallon of milk for brucellosis-free dairy cattle and £5 a head in addition to the hill cow and beef cow subsidy. Both these schemes are voluntary and aimed at attracting owners of herds with light to moderate infection to build up the numbers of brucellosis-free cattle. The subsidies are paid for a five year period and one of the conditions of the scheme is that animals found to be infected are slaughtered. In November 1972, compulsory slaughter will be introduced for infected animals in those areas now subject to movement restrictions—parts of north-west England, west Scotland, south-west Wales and the Isle of Wight. Farmers in compulsory eradication areas receive £30 in compensation for a slaughtered animal, provided that the free calf vaccination service has been used since July of this year. If the service has not been used, £15 will be paid, with market value being paid for any slaughtered "contacts" (the market value of a dairy shorthorn at the end of October was £111.50). Gradually the eradication areas will be extended until the disease can, it is hoped, be effectively wiped out.

Brucellosis is not a notifiable disease and it is therefore difficult to discover how many cases there are in Britain and how much it costs the country each year. A ministry survey of 3,000 herds in 1961 suggested that between 25 and 30 per cent of herds were infected but the survey only covered dairy herds and no figures for beef herds seem to be available. In 1967 it was estimated that 14 per cent of cattle would react to brucellosis tests and in August this year a Milk Marketing Board survey over six months, using milking tests and covering every herd with which the

board deals, showed that 28.5 per cent of herds contained reactors. A limited survey in Lancashire in 1970, also by the board, showed that 18 per cent of cattle reacted. There were 717 reported cases in humans in the United Kingdom and Eire last year, but it is thought that there were hundreds more either not reported or not diagnosed.

Estimates of the cost are equally vague, but a figure of £2 million to £3 million for the cost to the industry and of about £16 million to the country would seem reasonable, although the latter figure particularly is disputed.

The Ministry of Agriculture puts no official figure on the cost of eradicating the disease but something in the order of £100 million is a fair estimate. The size of this figure may account for the length of time it has taken Britain to begin eradication—some continental countries have virtually beaten the disease already, and a draft EEC regulation rules that after December 1975 no milk will be accepted from brucellosis-infected herds. The lion's share of the cost will be in compensation and incentives to farmers over the next few years. How long eradication will take depends upon a number of factors, not least the willingness of farmers to cooperate, but it is hoped to beat brucellosis in ten to fifteen years. This compares with the twenty-five years it took to remove bovine tuberculosis from 1935–60 at a cost of £130 million.

The eradication of the disease is necessarily a lengthy process as any attempt at rapid eradication would fail through a shortage of vets to carry out the work and would cause serious depletion of stocks. With a reserve of brucellosis-free animals, eradication can be managed without producing sudden shortages. The National Union of Farmers accepts that the ministry's scheme is the best approach although it is anxious that the compensation figures should be high enough—at the moment it feels that the £30 a head compensation for slaughter is not adequate because the cost of brucella-free stock to replace the slaughtered cattle is some 15 per cent higher than that for unproven stock.

Progress so far seems encouraging. The introduction of the incentives scheme has already involved 45,000 of the country's 200,000 herds and applications to join the scheme are running at about 1,500 a week. It is thought that these herds are those only lightly or moderately infected and of the 2 million or so animals tested from herds in the scheme there is a failure rate of just over 2 per cent. Due to the compensation to be paid for slaughtered animals it may be in the interests of those farmers with heavily infected herds outside the eradication areas to wait for compulsory eradication.

NEW WORLD

Conflicting Philosophies on Higher Education

by our Washington Correspondent

THE House of Representatives has done almost as much as the Senate to win friends in the colleges and universities. Like the Senate, it has departed from established traditions in voting for a huge infusion of federal money into higher education, although the proposals which have so far been agreed by each legislative body differ substantially about the method by which the largesse should be distributed. One common feature shared by the House and the Senate bills, however, is that both versions are likely to be opposed at least in part by the Administration.

The chief new provision contained in the bill which was passed last week by the House of Representatives is that federal grants would be distributed to every college and university in the United States. The bill, which was passed after four days and one night of debate, authorizes expenditure amounting to \$20,000 million over five years, including \$1,000 million a year on grants to institutions. Two-thirds of the institutional grants will be distributed on the basis of the number of students at each university and the remainder will be distributed according to the number of students at each institution receiving federal assistance grants.

In several respects, the bills passed by the House last week and by the Senate in August (see *Nature*, **232**, 518; 1971) represent differing philosophies on how to rescue colleges and universities from their present financial troubles. The Senate bill would set up a system of educational opportunity grants to each student, the amount of each grant depending on parental income, and it also provides for grants to institutions allotted on the basis of the numbers of students receiving these educational opportunity grants. The House bill carries these institutional support grants one stage further by also providing assistance to all colleges on the basis of their total student intake. It is that part of the institutional support provision that is likely to provide the chief stumbling block in the conference committee that must now sort out the differences between the two bills.

General institutional support is also likely to be opposed by the Administration, which favours institutional grants on the lines proposed by the Senate. The House rejected a move to knock general institutional support out of the bill, however, by a vote of 310 to 84, and the representatives of the House in the conference committee now have a clear

mandate to keep the provision in the bill. The amendment to delete general institutional support from the bill was proposed by John N. Erlenborn, who argued that there is not enough evidence of financial hardship in every college and university to warrant grants to every institution, while Edith Green, chief proponent of the bill, argued that colleges and universities in the United States are "facing the greatest financial crisis they have ever seen".

In spite of their concern over the financial state of American universities and colleges, however, members of the House of Representatives removed from the higher education bill a provision for bailing out the most severely affected institutions with emergency aid. The bill originally proposed an authorization of \$150 million this year and the same amount next year for such institutional support, but this was struck out on the grounds that the Commissioner of Education could not determine fairly which institutions should receive the support.

The House bill also extends the education opportunity grant system, raising the grant level from \$1,000 a year to \$1,500 for needy students, and extends the guaranteed student loan programme. The bill also bans discrimination on the grounds of sex for any institution receiving federal grants, but an amendment making the ban inapplicable to student admissions was carried by a small margin on the grounds that it would effectively impose federal admissions policies on the universities.

Other measures included in the bill are:

- An authorization of \$5 million in 1972 for federal aid to college and university libraries, increasing to \$40 million in 1976.
- Authorization for student loans at the rate of \$425 million in 1972 rising to \$675 million in 1975 and 1976.
- The setting up of a new National Institute of Education in the Department of Health, Education and Welfare, on the lines of the Senate-passed measure. This proposal, supported by the Administration, would have the primary function of conducting educational research.
- The bill also calls for the setting up of a special commission on the financing of postsecondary education, to report by June 30, 1973.

Although both the Senate and House-passed bills contain much that is accept-

able to the Administration, they will both stick in the throat of the Office of Management and Budget because each raises the federal higher education bill much above its present level. The Senate version, for example, asks for \$18,000 million over the next three years, while the House version asks for some \$20,000 million over the next five years. Present funding runs at about \$1,300 million a year.

Whatever the outcome of the conference, which will not take place for some time because of the difficulty in sorting out the areas in which the two massive bills do in fact differ, it is clear that the final measure will contain some provision for direct institutional support. The Senate wants such support distributed to colleges and universities whose students receive federal aid, while the House favours the major part of the federal grant money going to all universities on the basis of their total intake. Either provision would, however, be a landmark in higher education in the United States.

CANCER RESEARCH

NIH wins Round Two

by our Washington Correspondent

IT now seems almost certain that the House of Representatives will vote to give the National Cancer Institute a budget of \$1,600 million during the next three years, but to keep it firmly a part of the National Institutes of Health. This is in sharp contrast to the Senate which agreed last July, by a vote of 79 to 1, to set up an independent agency devoted to finding a cure for cancer, and it means that the crucial decisions on the future organization of cancer research in the United States are likely to be taken behind the closed doors of a conference committee.

The mood of the House of Representatives can be judged by the fact that the House Interstate and Foreign Commerce committee last week agreed by a vote of 26 to 2 to send to the floor of the House the bill introduced recently by Paul G. Rogers with the relatively minor amendments tacked on by the subcommittee on Public Health and Environment (see *Nature*, **233**, 228; 1971). The support for Rogers's bill in the Commerce committee was in fact so strong that a move to amend it by striking out the word-

ing and inserting the Senate-passed version attracted only four votes. But perhaps the strongest demonstration of support lies in the fact that no Republican member of the Commerce committee voted to substitute the version passed by the Senate, in spite of strong pressure from the White House. In fact, Elliot L. Richardson, Secretary of Health, Education and Welfare, took the trouble to write to every member of the committee urging them to support the Senate version.

Members of the House of Representatives will therefore be faced with a bill designed to give cancer research a greatly increased budget, and, like members of the Senate, they will not run the risk of seeming to vote against cancer research by opposing the bill. The chief challenge to the Rogers bill will therefore come from amendments designed to replace the bill with the Senate version. Mr Brock Adams, who voted against the Rogers bill in the Interstate and Foreign Commerce committee last week, is intending to introduce such an amendment from the floor of the House, and he will have the support of the White House lobbyists.

Even those who support Mr Adams's amendment, however, admit that it stands little chance of succeeding since the tide is now running so strongly in favour of keeping cancer research within the National Institutes of Health. Another reason why the amendment will not pack a very heavy punch is that the White House is in an embarrassing position over the two bills, and will find it difficult to lobby for the Senate version with any conviction. The problem is that the bill passed by the Senate is very similar to a bill introduced by Senator Edward M. Kennedy. The Administration opposed Kennedy's bill on the grounds that it might lead to the break up of the National Institutes of Health, but later came to a compromise which effectively put the Administration's name on Kennedy's proposals. The White House is therefore now urging Congress to turn down a bill whose objectives it once supported. The Administration has, however, been consistent in its wish to give public voice to its desire to aid cancer research—a great asset in election year.

SPACE

No Strings Attached

by our Washington Correspondent

ONE of the chief rocks on which European cooperation in space research has foundered in the past year or so has been removed by the US State Department. It was announced last week that NASA will launch European scientific

and technological satellites without assurance of European collaboration in the space shuttle programme, and the US government is also prepared in principle to launch telecommunications satellites for European countries.

The State Department's offer, which was outlined in a letter from Under-Secretary of State U. Alexis Johnson to M. Theo Lefevre, chairman of the European Space Conference, may be sufficient to tempt European governments to abandon the Europa III launcher (which is designed to launch the type of satellites on which the US government has now given an assurance), thus freeing European finances for participation in the shuttle programme. The letter was sent in September, but was not made public until last week.

The new attitude of the US government marks a considerable shift in policy. For one thing, discussions of US launch policy have for the past year been underpinned—tacitly if not explicitly—by the assumption that provision of US launchers would be contingent on European cooperation in the space shuttle programme. The US government has also been chary in the past of giving assurances on the launching of telecommunications satellites that may in any way compete with Intelsat. That, at least, was one of the chief reasons advanced in Europe for pressing ahead with Europa III.

The US government is not, however, prepared to ignore Intelsat in the hope of promoting closer accord with Europe on space research, for the assurance on launcher provision is conditioned by several clauses designed on the face of things to protect Intelsat interests. Briefly, the United States will provide launch facilities for communications satellites approved by Intelsat, but blanket launch assurance cannot be given for satellites that are not supported by Intelsat.

According to the Intelsat agreement signed earlier this year, the consortium must give its advice on telecommunications satellites flown independently by member countries, the chief consideration being the extent to which the proposed satellite will compete commercially with those sponsored by Intelsat. The US government is now saying that it will launch European satellites that gain Intelsat approval, or which have been supported by the US within Intelsat. Since it is highly unlikely that satellites backed by European and United States interests would fail to gain Intelsat approval, the agreement boils down essentially to the question of US support. The agreement comes very close in practice, however, to being a complete assurance of the availability of US launchers for all types of satellites.

Whether or not such an assurance will

help to sew together the tattered fragments of space collaboration in Europe is open to question, but the announcement has at least laid the basis for future negotiations between the governments of Europe and the United States. Already, the State Department has suggested that discussions should not be confined to collaboration on the shuttle, but that they should be broadened to include all kinds of space research, and two weeks ago a NASA official was dispatched to Paris to tell the European Space Conference of NASA's plans for the next ten to fifteen years. If the launch assurance is sufficient to persuade European governments that Europa III is no longer needed, such discussions would have a much greater chance of success.

NUCLEAR WEAPONS

Lessons from Cannikin

from a Correspondent

WHAT the US Atomic Energy Commission scientists learnt from the Cannikin test on the Island of Amchitka will probably never be known, being apparently vital to national security. The lessons for all other participants in the affair are more obvious.

The drama associated with Cannikin was appropriate to a Moon shot. Environmentalists have fought during the past week a desperate court action to have papers on the environmental impact of the test released and then to use them in demonstrating that there were significant risks of earthquakes, tidal waves and radioactive leakage. On the very morning of the test, the Supreme Court sat to hear arguments on the environmental impact, and voted 4 to 3 that the test could proceed. Thereafter the AEC, clear of its last hurdle, went ahead and fired the shot which predictably triggered no earthquakes or tidal waves. It is too early to tell whether there will be any radioactive leakage, but there appears to be no concern about it.

One of the most remarkable and least noted features of this test is that in the fifties and early sixties the United States fired such shots in the atmosphere with impunity. The American public has come a long way in its attitudes since then. Unfortunately, the issues on which the AEC were challenged were in the opinion of many flimsy. It is of course an old political trick to steer clear of the central arguments and try to make the peripheral questions seem more important. The AEC did not even have consciously to pursue this policy; the environmental question was the only one which generated a significant amount of heat. To be sure, there is never a zero risk of environmental damage and no scien-

tist has come out with a statement that environmental arguments are nonsense, but it appears to have been the considered opinion of practically all those who studied the problem in a detached manner that the risks were very slight.

What environmentalists would desperately have liked would have been a concerted declaration from scientists warning of dangers. No such statement was forthcoming. It is indeed conceivable that the environmental movement has been harmed by the Cannikin affair. Its members may have learned the hard way that scientists with some sympathy for the movement can be alienated by a stance that scientists could not maintain. They may have lost some credibility with those middle Americans who are unimpressed when the Doomsday does not show up to order. The first callers to radio talk shows immediately after the blast were saying, "Well, what was all the fuss about then?" They may also have learnt that when dealing with politicians they are talking to pragmatic people for whom a 10 per cent, let alone 1 per cent, chance of catastrophe is acceptable. The environmentalists have to walk on a tightrope above scientists, lawyers, politicians and the general public. The Cannikin affair may have helped them to learn new tactics.

Of course, what Cannikin was really about was national security. If a good number of Senators had banded together early enough to express disbelief that this one explosion was more vital to national security than good relations with Canada and Japan, the soft centre of the Administration's argument would have been revealed. Fresh from previous insults from the new Nixon, these two countries in particular were bitter about the test and are going to be more difficult to deal with in the future as a result of it, earthquake or no. Whether the milliseconds worth of X-rays is more valuable than good relations with two allies is much the most debatable issue in the whole affair.

MARINER 9

Into Orbit around Mars

THE arrival of Mariner 9 in the vicinity of Mars today will open up a new chapter in the exploration of the solar system. If the craft successfully enters orbit around the planet, its sensors should be able to resolve some of the questions concerning the environment of Mars which have been puzzling astronomers for centuries. A spokesman for NASA predicts that there will be "a veritable information explosion about the Mars terrain, climate and atmosphere", and about its two moons Phobos and Deimos. The exchange of information obtained by Mariner 9 and

by the two Russian probes also now in the vicinity of Mars will undoubtedly add to this explosion—it may be that one or both of the Russian probes is planned to attempt a landing on the surface of the planet.

Some indication of the importance of these missions is provided by the way in which the earlier Mars flyby missions changed man's ideas about the planet. It is now clear that the planet is cratered in a way similar to the Moon but that the craters are weathered and that there are also regions of apparently recent activity. Apart from straightforward photography which should produce 5,000 pictures—the 1969 mission produced 205 pictures and the 1965 mission only 22—the spacecraft carries an infrared radiometer, an infrared spectrometer and an ultraviolet spectrometer, each mounted to point at the object being photographed. In this way, the temperature, terrain and atmospheric conditions in each area pictured will be recorded.

The most interesting phenomena yet discovered on Mars are the clouds,

hazes, bright spots and dust storms. A specific objective is the study of the wave of darkening observed seasonally. The spacecraft is arriving when this darkening is at its seasonal peak in the southern hemisphere. In some quarters this has been attributed to a spread of moisture from the poles with the onset of spring. A more widely held belief is that the wave corresponds to the removal of light coloured dust. Hopefully this conflict together with many others will be resolved in the near future.

One question that is not expected to be answered yet concerns the possibility of life on Mars. At present there seems little chance that any complex life forms can exist on the planet, and Mariner 9 would be unable to detect primitive life forms even at closest approach (800 miles). Recently, however, prebiotic molecules have been synthesized in the laboratory under conditions like those on Mars and it is possible that Mariner 9 could provide evidence that biological molecules are present in the Martian atmosphere.

Short Notes

Uranium Enrichment

REPRESENTATIVES of the Australian, Japanese and Canadian nuclear industries met in Washington last week with officials of the AEC and the Department of State to discuss the possibility of using US gaseous diffusion technology in multinational uranium enrichment plants. This was the first tangible sign of cooperation since the AEC announced in July that it was prepared to discuss the possibility (see *Nature*, **232**, 366; 1971). Representatives of several European countries will go to Washington for similar meetings on November 16.

It is believed that the talks centre on the possibility that two enrichment plants will be built and operated on a multinational basis—one by Japan, Canada and Australia, and the other by European interests. The chief points under discussion are safeguards to prevent nuclear material being diverted to weapons production, and the US government also wants an assurance that its enrichment technology secrets will not be able to leak out to other countries.

DDT

THE Environmental Protection Agency has decided not to suspend all uses of DDT while cancellation proceedings are going on. This allows the pesticide to be manufactured and sold throughout the United States while the EPA decides whether or not to prohibit its sale across state lines. Announcing this decision last week, Mr William D. Ruckelshaus,

Administrator of the EPA, pointed out that the cancellation proceedings must be completed by March 1972, but will probably be concluded long before then. He suggested that no appreciable difference would result from any ban that could be imposed before that date.

Unemployed Chemists

EVEN those chemists who were lucky enough to find jobs waiting for them when they left the universities this summer have good reason to wish that they had graduated in a sunnier economic climate. According to a survey carried out by the American Chemical Society, their average starting salary was some 7 per cent lower than starting salaries a year ago—and, taking into account the fact that the cost of living increased by more than 4 per cent during the year, this means that starting salaries dropped by about 11 per cent compared with 1970.

But, while new graduates in employment may have to take in their belts by half a notch, many of their colleagues are much less fortunate. The ACS survey found that unemployment among newly-qualified chemists doubled from 5.1 per cent in 1970 to 10.3 per cent in 1971. First degree graduates suffered an unemployment rate of 11.6 per cent, while those with master's degrees reported a rate of 8.8 per cent and PhDs 6.1 per cent. Moreover, those who left the universities with a degree in chemical engineering found the job market even less hospitable—an alarming 12.8 per cent reported that they had not found a job by the end of the summer.

NEWS AND VIEWS

Links Between Chains

It took probably a century of man years to decide whether myosin was two or three stranded. No sooner had the dust of that wrangle settled than another argument was on: low-molecular weight proteins were apparently shed by the myosin molecule, under conditions that led always to denaturation, or at least to loss of ATPase activity. Were these "light chains" real components of the functional molecule, or merely bound impurities? How many species are there, and in what proportion are they present on the myosin? Are they involved in the ATPase function? Does their heterogeneity perhaps arise from different populations of fibres? It may be hoped that two meticulous studies in this edition of *Nature* will help shake off the incubus of numerical uncertainty that has so long dogged the field.

Lowey and Risby (page 81) have addressed themselves to the general problem of the estimation of protein components in mixtures by gel electrophoresis. The light and heavy chains cannot be compared on a single polyacrylamide gel, because the light chains diffuse too rapidly in a gel of low concentration, whereas the heavy chains will not penetrate a gel of high concentration. Comparisons have therefore to be made between the components in separate gels, and a protein of intermediate molecular weight, tropomyosin, is added to the mixture to provide a common concentration standard for both gels. The linearity of integrated staining intensity with the quantity of protein applied to the gels was demonstrated, and the same relative concentrations of components were obtained with the use of two different protein stains. Moreover, when separated light and heavy chains at defined concentrations, based on nitrogen determination, were run on gels, the staining intensities were found to reflect faithfully the relative concentrations of the different components—a result which, not necessarily general, should give comfort to workers in other branches of protein chemistry. Using molecular weights based on mobility in gels containing the detergent SDS, the stoichiometry of all the components could then be evaluated.

The upshot is that in three fast-muscle preparations, from rabbit and chicken, each molecule of myosin gives rise to four light chains, two of a species of 18,000 molecular weight and two others of 16,000 and 20,000 respectively. It is the first kind that are liberated by treatment with a thiol-specific reagent (Ellman reagent), and their loss is not accompanied by any major change in any measured properties of the myosin, ATPase included. Slow muscles give a quite different pattern: two species of light chain are resolved, which do not correspond to any of the above components, have molecular weights estimated at 20,000 and 27,000, and are present in a ratio of two moles of each per mole of myosin. The results in terms of the number of components, and their differences, as between skeletal and cardiac myosins, are, it is agreeable to report, in substantial agreement with a recent parallel study by Sarkar, Sreter and Gergely (*Proc. US Nat. Acad. Sci.*, **68**, 946; 1971).

In red muscle it looks as though both types of myosin may be present, with five light chain components clearly discernible. The identical patterns from the muscles of

two animals are particularly satisfactory features of these results, encouraging, as they do, the confidence that the stoichiometry is correct, and the heterogeneity not adventitious. Moreover, the difference between the light chains from fast and slow muscles, allied to their apparent inseparability from the myosin ATPase activity, raises the alluring possibility that they in fact regulate this function.

In the companion article, Weeds and Pope (page 85) examine the chemical relation between the various light chains. The identifying feature which they have selected is the sequence of the proteolytic fragments containing thiol groups, because these can be labelled with an isotopic alkylating agent and can thus readily be identified after electrophoretic separation of a chymotryptic digest. By this technique Weeds previously demonstrated that the inessential light chain component released by Ellman reagent contains two unique thiol peptide sequences, whereas the remaining light chains possess only one between them. Working this time with prime beef, Weeds and Pope find that cardiac (slow muscle) myosin light chains give rise in all to three different thiol peptides, one of which, a fragment of thirteen residues, is identical to the single thiol sequence present in the ATPase-associated light chains of skeletal muscle myosin.

Now the cardiac myosin light chain preparation contains two different components. Complete separation of these has not been achieved, but it seems certain that one (the larger, with molecular weight 27,000) is responsible for all three thiol peptides, whereas the smaller, of 20,000 molecular weight, most probably possesses no thiols. Sheep heart muscle myosin contains similar light chains, which give rise to the selfsame thiol sequences as those of beef heart myosin. Rabbit cardiac myosin is evidently again similar. The significance of the long common thiol peptide segment between light chains from cardiac and skeletal muscle is not obvious, for in other respects, including the molecular weight, the chains are distinctly different.

Weeds and Pope add that their unpublished results on fast-twitch and slow-twitch muscles of the cat show light chain patterns which are respectively similar to skeletal and cardiac muscle myosin. These results point once more to an enzymatic regulatory function for at least some of the light chains. The heterogeneity remains a curious feature; it may be noted that a current report (Hale and Beecher, *FEBS Lett.*, **18**, 245; 1971) has started once more the hare of heterogeneity of the heavy chains. Gel electrophoresis of the denatured chains in concentrated urea solution in the hands of these workers produces two components in equal concentration. They have done their best to control the rather extensive possibilities of chemical or physical artefacts, but workers in the field may wish to reserve judgment.

If these results are taken at face value, however, they raise now the question of whether the two components represent different halves of all myosin molecules, or different myosin populations. Much work remains to be done on myosin, but there should be no mistake about the importance of establishing the composition of the molecule in definitive terms.

SEXUAL SELECTION

Mating Preferences

from our Population Genetics Correspondent

DARWIN's notebooks show that he deduced his theories of sexual and natural selection at about the same time. Sexual selection was briefly mentioned in *The Origin of Species* and discussed in detail in *The Descent of Man* published in 1871. Although direct observations of natural selection were first made around 1900, it is only in the past two decades that sexual selection has been observed experimentally. Sexual selection occurs if there are variations in the chances of mating. It usually affects the males and has always been accepted as the cause of the evolution of the special weapons such as horns and spurs used in the fighting between the males of certain species when they collect their mates.

Darwin thought that the females would also have mating preferences for particular male characteristics, especially those involved in mating displays. For a long time many naturalists refused to accept this possibility of female choice in the selection of mates, and it was left to the population geneticists to demonstrate by experiment that there were female mating preferences for males with particular genotypes. If a female *Drosophila* is placed in a mating chamber with one wild type and one white eyed male, observations show that the female will usually mate with the wild type male. But a male given a choice of wild type and mutant females usually shows no preference and matings take place with both females with equal frequency.

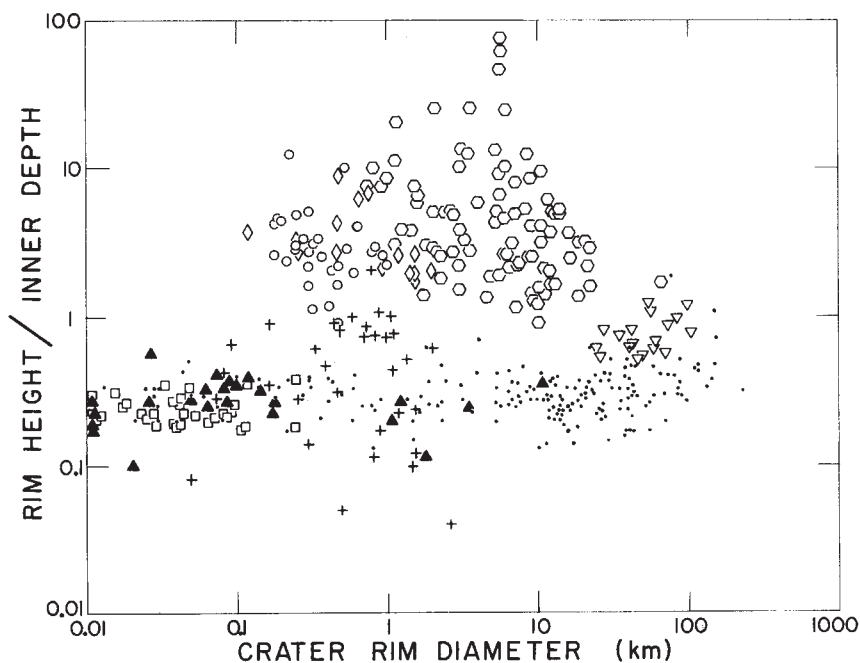
In experiments with *Drosophila* Ehrman showed (*Amer. Nat.*, **101**, 415; 1967; *Genet. Res.*, **11**, 135; 1968) that the mating success of the males could be frequency dependent: the females had a tendency to mate with those males who possessed the rarer genotypes for particular chromosomal inversions. In the latest issue of *Evolution* (**25**, 265; 1971), Faugères, Petit and Thibout describe experiments to analyse different components of sexual advantage. They studied the sexual vigour of the males (their ability to inseminate several females) and their success in sexual competition (the female preference for particular genotypes). They used mutant white *Drosophila melanogaster* and various wild type strains and their hybrids. Sexual competition was studied by giving the males or females either a limited choice of two individuals of the opposite sex, white and wild type, to mate with, or a multiple choice with ten flies of one sex and five of the other in the mating chamber. They then observed the copulations that took place. Sexual vigour was measured

by the number of females which a male could inseminate in 24 hours.

As in previous experiments, the sexual competition affected only the males. The white males were always at a disadvantage. On the other hand, in sexual vigour, the hybrids, white and wild type, were superior to the pure strains, white and wild type. This shows that different factors are responsible for sexual selection and sexual vigour. Genotypes advantageous in sexual selection may not be advantageous in sexual vigour. Faugères, Petit and Thibout point out that the ethological factors that determine the advantage in sexual selection could be masked by other factors causing

superior sexual vigour. This may affect not only sexual selection but also sexual isolation. When strains evolve sexual isolation (as in the experiments recently reported by Dobzhansky and Pavlovsky in *Nature* (**230**, 289; 1971), a particular strain evolves a mating preference within itself so that eventually matings take place only between members of a particular strain. This could be prevented if the male hybrids had a superior sexual vigour and could inseminate more females although they were less likely to be chosen as mates. If the hybrids are inferior in sexual vigour, however, the mating preferences will continue to evolve and will eventually produce sexual isolation between

Craters on the Earth and Moon



IN next Monday's *Nature Physical Science* R. J. Pike of the US Geological Survey, Menlo Park, California, classifies lunar craters according to the ratio of the external height of their rims to the interior depth of the crater floor. For normal lunar craters the ratio is usually well below unity. In other words, the floors of lunar craters tend to be below the general level of the area in which they are situated.

There are exceptions of course. Lunar craters that have been flooded with mare material have ratios of external height to interior depth of about unity, and lunar craters on cones have ratios in excess of unity.

In the figure Pike has plotted the ratio of external height to interior depth against the diameter of the crater (from rim crest to rim crest) for various kinds of lunar and terrestrial craters. The dots are what he calls "normal" rimmed lunar craters, the open triangles are lunar craters filled with dark mare

material, the lozenges are summit craters on lunar domes, the hexagons are terrestrial calderas, the circles are terrestrial cinder cones, the crosses are terrestrial maars (volcanic explosion craters), the squares are experimental explosion craters and the solid triangles are meteorite impact craters on the Earth.

Pike points out that the ratio of external height to interior depth, as plotted on the figure, tends to distinguish calderas, cinder cones and lunar dome craters from normal lunar craters, maar craters, meteorite craters and experimental explosion craters, and he interprets this distinction as indicating two contrasting modes of origin. Calderas, cinder cones and, presumably, lunar dome craters are essentially constructional features formed during a series of volcanic eruptions, whereas the second group of craters seem to be characterized by formation during single catastrophic explosive events.

the strains. These would therefore have become separate species as in Dobzhansky and Pavlovsky's experiments.

Sexual isolation can thus be produced by mating preferences in favour of two or more different genotypes. This is the sexual equivalent of disruptive natural selection in which different genotypes are favoured in different environments. When this occurs, the hybrids may indeed be disadvantageous, being adapted to none of the environments as well as each particular strain. This disruptive natural selection will thus allow the evolution of mating preferences in each strain, and therefore produce what may be called disruptive sexual selection.

ENZYMES

Orbital Argument

from our Molecular Biology Correspondent
SOME months ago I described what seemed at the time the obliteration by Bruice and his colleagues of Koshland's "orbital steering" theory of enzymatic catalysis. Those people who enjoy the cathartic effects of an intellectual punch-up will be delighted to see that Koshland has not taken the assault on his brainchild lying down, and has come storming back in the current issue of the *Proceedings of the US National Academy of Sciences* (Dafforn and Koshland, *Proc. US Nat. Acad. Sci.*, **68**, 2463; 1971).

The argument really hinges on how close must be the angular restriction on the line of attack of one reactant by another in order to bring about a rate enhancement of a good many orders of magnitude. Bruice *et al.* concluded from some very straightforward calculations of orientation factors that large enhancements demand an angular precision of the order of 0.1° , which would fall within the range of normal vibrations. Dafforn and Koshland criticize the logic which leads to this conclusion, first of all on the grounds that the orientation of only one of the reactants was considered, and secondly that the calculation pertains to a substantially higher rate increase than it is necessary to find, given that there is some choice in distributing rate-enhancement factors between different possible causes. Adjustments for these considerations, say Dafforn and Koshland, bring the angular restriction up to the altogether more reasonable level of 10° —though this is still not very different from room-temperature vibrations. They go on to show that if one computes the entropic effect of rotational immobilization of the reactants, that is to say one locks them in a favourable orientation for attack, one can come by an adequate enhancement factor. They also then calculate the change in the energy of the

transition state, corresponding to a given change in the angle of approach, using a simple harmonic oscillator model with a reasonable value for the bending force constant. Depending on the force constant chosen, the rate factor falls off fairly rapidly with angular distortion.

What then are we to believe? It is clearly possible for theoreticians to hurl formulae at each other, which prove their opposing cases with comparable conviction, and presumably to their entire satisfaction. It is perhaps not unfair to take the view that the onus of persuasion is on Koshland, because his notion does represent a departure from implicit chemical thinking. His classical calculations inevitably make several considerable numerical assumptions about the nature of the transition state. Moreover, there are the quantum mechanical calculations of Port and Richards to be considered (*Nature*, **231**, 312; 1971).

Storm and Koshland, in their original formulation of the "orbital steering" concept, gave as evidence in its favour the relative rates of intramolecular esterification in a set of compounds in which the reacting carboxyl and hydroxyl groups were positioned with

different degrees of rigidity, and also the relative rate of thioester formation when sulphur was present in place of oxygen. Port and Richards have simply calculated the overlap integrals for the orbitals involved in the reaction, on the grounds that these must be expected to reflect the relative reaction rates, if the orientation factor is indeed dominant. As one might intuitively expect for the set of components, on the grounds of the diffuseness of molecular orbitals, the variation in overlap integrals is slight. Port and Richards concede that more refined calculations would be both possible and desirable, but their results as far as they go certainly argue against the importance of the critical angular orientation factor.

At the very least then one may say that Koshland's case is not proven, though of course the strategy of destruction is always much the easier in such a situation. In Koshland's favour it might be argued that orbital steering should not be discarded until a good alternative can be found, and Koshland rejects the proximity effect (that is the translational, as opposed to rotational immobilization of the reactants, in juxtaposition at the active site) as a

Defective Mouse Sarcoma Virus

As RNA tumour virologists learn progressively more about the biology of the mammalian and in particular mouse sarcoma and leukaemia viruses, it is becoming increasingly apparent that these viruses are capable of essentially the same range of interactions with their host cells as their avian counterparts, the Rous sarcoma viruses and avian leukosis viruses. In *Nature New Biology* next Wednesday, for example, Gazdar, Phillips, Sarma, Peebles and Chopra describe the isolation and properties of a noninfectious mouse sarcoma virus which is reminiscent of the noninfectious Rous sarcoma viruses produced by certain chick cells transformed by Bryan high titre Rous sarcoma virus in the absence of an avian leukosis helper virus.

Gazdar *et al.* noticed that two lines of hamster cells derived from sarcomas induced in hamsters by mouse sarcoma virus liberated typical C-type virus particles with all the biophysical parameters of the sarcoma leukaemia viruses. These virus particles, in spite of the fact that they are liberated from hamster cells, proved to cross react with antisera against the mouse sarcoma and leukaemia viruses but failed to cross react with antisera directed against hamster leukaemia viruses. In short, the virus liberated by the hamster cells is antigenically related to mouse, not hamster, C-type viruses.

Is the virus from the hamster cells in-

fectious? All the attempts which Gazdar and his colleagues have made to transform mouse and hamster cells growing in a variety of culture conditions have failed and neither does the virus induce sarcomas or leukaemias when injected into newborn rats, mice or hamsters. Gazdar *et al.* were, however, able to prove that these apparently noninfectious mouse C-type virus particles do indeed contain that part of the mouse sarcoma virus genome which is responsible for transformation and sarcomagenesis by sedimenting this virus with mouse leukaemia virus. During such co-sedimentation the two viruses are presumably physically fused together, for the pelleted virus particles will transform cells and at low efficiencies induce sarcomas. After sedimentation alone neither the noninfectious sarcoma virus nor the leukaemia virus has these properties.

Gazdar and his colleagues conclude that the virus which they have isolated from these hamster cells contains the mouse sarcoma virus genome but lacks helper leukaemia virus of either mouse or hamster origin. The parallels and differences between this virus, another defective mouse sarcoma virus isolated recently by the same group (*Proc. US Nat. Acad. Sci.*, **68**, 1520; 1971), and the defective Rous sarcoma viruses discovered and studied by Weiss and the Hanafusas deserve and are no doubt receiving further attention.

candidate in quantitative terms. On the simplest argument, based only on translational entropy, the proximity factor on the reaction rate should be only a matter of 55. Page and Jencks (*Proc. US Nat. Acad. Sci.*, **68**, 1678; 1971) have now argued, on the basis of calculations of translational and rotational entropy changes on binding the reactants, that the effect may be very much greater, and that effective reactant concentrations of 10^8 molar can be achieved at an active site. On this view, the enzyme operates primarily as an entropy annihilation machine to produce the required rate enhancement. Dafforn and Koshland take issue with Page and Jencks, and trade approximation for approximation, and entropy unit for entropy unit. They also make short work of Reuben's argument (*ibid.*, 563) regarding the longer sticking time of reactants on the active site, compared with free solution collisions, by pointing out that this is compensated by a correspondingly lower collisional frequency.

The argument will clearly continue, and the difference of view between Koshland and Jencks will readily lend itself to semantic hair-splitting. Whether or not orbital steering is now a dead duck is a matter on which most enzymologists will make up their own minds. There is no doubt, however, that it has sparked off an uncommonly interesting argument.

GENE EXPRESSION

Modifying Transcriptase

from our Cell Biology Correspondent

NOT so long ago when sigma factors were at the height of fashion they were being invoked whenever questions of the regulation of gene expression were being discussed. The simple notion that core DNA dependent RNA polymerase (α, β, β'), a nonspecific machine for catalysing the polymerization of ribonucleoside triphosphates into RNA, might specifically be programmed by a set of different sigma factors each of which restricted transcription to a particular set of genes could be adapted to explain almost any pattern of regulated gene expression. This idea has, however, lost much of its appeal, not least because the putative sets of sigma factors have not materialized. Indeed, in perhaps the most intensively investigated system in which there is a regulated change in the pattern of transcription—the replication of phage T4 in *Escherichia coli*—it is modifications of the polypeptide chains which comprise the cell's core enzyme and factors involved in termination that seem to play a crucial part in switching transcription on and off.

As Schachner and Zillig report

(*Europ. J. Biochem.*, **22**, 513; 1971), the core DNA dependent RNA polymerase which is isolated from *E. coli* some 10 minutes after infection with T4 differs in all its subunits from the core enzyme of uninfected cells. Fingerprints of tryptic peptides from modified (post-infection) and unmodified (pre-infection) core enzyme reveal the following: after infection the α chain of the core enzyme apparently gains additional amino-acids and, as Goff and Weber have reported, an adenylic acid residue; the β' chain is modified such that it yields on tryptic digestion an additional tyrosine containing peptide and one of the histidine containing peptides has an altered mobility; finally, the β chain from modified core yields a significantly different set of histidine and tryptophan containing peptides.

From T₄ to Man

WHEN Alberts and Frey (*Nature*, **227**, 1313; 1970) described the properties of the gene-32 protein in T₄ bacteriophage it was hailed as a milestone in the understanding of both genetic recombination and DNA synthesis. Hotta and Stern (in next Wednesday's *Nature New Biology*) now announce that proteins with very similar properties to that of this T₄ protein can be isolated from spermatocytes of three mammalian species, namely rat, bull and man. Evidence that meiotic cells of lily contain this DNA-binding protein has already emanated from Hotta and Stern's laboratory (*J. Cell Biol.*, **47**, 91a; 1970), so that it seems highly likely that the protein may occur universally.

On the basis of various bits of information which have become available over the past couple of years, Hotta and Stern have pieced together their model for recombination. The picture which is emerging is of two types of pairing during meiosis, one occurring at zygonema and resulting from delayed replication of small stretches of DNA such that only short segments of the chromosome are paired; this would be most likely coordinated with synaptonemal complex formation and the stabilization of the chromosome pairs. It is suggested that the second type of pairing would follow this initial alignment, with nuclease and other enzymes producing single stranded regions of DNA. The type of recombination which other authors have described might then occur within these regions. Hotta and Stern think that the DNA-binding protein may play some part in either of these two forms of association but because of the widespread distribution of the protein it seems likely to be of important functional significance for meiosis generally.

In short, every polypeptide chain in the core enzyme is altered after T4 infection and Schachner *et al.* (*ibid.*, 520) attempted to correlate these changes with changes in the pattern of transcription of the T4 genome. The chief events are, first, the switching off of host RNA and protein synthesis, which occurs in the absence of protein synthesis and can be brought about by allowing phage ghosts to adsorb to the cells. Once this step has occurred the immediate early genes, then the delayed early genes and finally, after DNA replication, the late genes of the phage are transcribed. This regulation is not the result of the sequential modifications of the three species of polypeptide chains in the core enzyme, for within 4 minutes of infection, before delayed early and late RNAs appear, all three chains have been modified. Modifica-

The protein was isolated from meiotic prophase nuclei, assaying for DNA-binding activity in much the same way as Alberts's T₄ protein and comparing the activity with that of the other spermatocyte nuclei. On fractionation, the greatest amount of the mammalian DNA-binding protein was found in exactly the same band as that in which the lily DNA-binding protein is found and, more important, the properties of all proteins, T₄, lily and the mammalian ones, seem to be identical.

All of the DNA-binding proteins are capable of renaturing denatured, single stranded DNA at a very fast rate and all of them will bind very similar amounts of DNA. Thus, in spite of the very limited purification which centrifugation achieves, the ratio of DNA bound to protein is approximately the same in all instances and suggests that the proteins have very similar DNA binding capacities.

Moreover, the elution profiles from both DEAE cellulose and from DNA columns have almost identical patterns, so that it would seem that the protein is a distinctive and general feature of eucaryotic nuclei. Because Hotta and Stern only find appreciable quantities of the protein in prophase nuclei with the other spermatophyte nuclei and the nuclei of somatic tissues containing insignificant amounts, it is not unreasonable to assume that the protein plays an important part in some facet of meiosis, and in view of the T₄ evidence, neither is it unreasonable to suppose that it has some recombinogenic activity.

This is interesting information and no doubt the next step in the confirmation of the protein's role will come from a determination of whether cells which undergo induced somatic recombination have elevated levels of the protein.

tion of core enzyme thus seems to be a very early event in T4 replication.

What then causes the switch from immediate to delayed early gene transcription and later to late gene transcription? From comparisons of the pattern of phage RNA made *in vivo* in the presence and absence of protein synthesis and the presence or absence of phage DNA replication (replication is blocked with certain mutant phage) and the ability of cell extracts to make RNA *in vitro* Schachner *et al.* suggest that one of the proteins specified by the immediate early genes is an anti-terminator factor which allows RNA polymerase to read through from the immediate early to the delayed early genes. Similarly, in the absence of DNA replication immediate early and delayed early RNA, but no late RNA, is made, and again Schachner *et al.* interpret this in terms of failure in normal termination, rather than initiation mechanisms, which stocks up the enzyme at the end of the delayed early genes. In short, regulation of termination may be at least as important as regulation of initiation in this particular example of controlled gene expression.

mittee of the Royal Society, presided over the session.

Dr Norman Simmons (Ministry of Defence) opened the meeting with a presentation on the state-of-the-art in remote sensing from space. Dr Simmons was particularly concerned to point out the limitations in the resolution of existing systems and the likely extent of possible improvements. At present a ground resolution of 50 m can be considered better than average and the systems fall rather short of the resolutions demanded by topographic mapping. Dr D. Parry (Hunting Surveys) followed with a description of an analysis of an oblique, hand-held photograph from Gemini VI of part of West Pakistan. Field surveyors had already worked in the area, mapping vegetation, soils and land use. By following a systematic procedure of tonal classification, pattern interpretation, collation of information available before field work and a subsequent comparison of these maps, Parry was able to demonstrate that, had the field surveys been pre-

sented with the photography beforehand, they could have significantly reduced the density of field sampling. This was in spite of considerable blurr, a varying scale in the perspective view and a predominantly blue coloration in the film.

Dr J. A. Jones (Experimental Cartography Unit, Royal College of Art) then spoke about plans to analyse the mappable content of ERTS-A imagery by computerized comparison between the imagery and previously mapped "ground truth" data on vegetation, geology, land use, soils and topography. The "ground truth" data consist of digitized field maps, that is, strips of identification codes and coordinates filed on magnetic tapes, and are the raw material for current experiments in structuring a computer storage and retrieval system for cartographic information. The experiment is centred on the Upper Thames Valley.

Dr J. Norman (Imperial College, London) attempted to forecast the value of ERTS imagery for geological pur-

REMOTE SENSING

Using ERT Satellites

from a Correspondent

INTEREST in the potential of remote sensing techniques has been slow to develop among field scientists in the United Kingdom compared with their colleagues in North America. This can very largely be explained by the relatively high cost of remote sensing operations, the scarcity of suitable sensors in the UK, the geometric limitations of many existing sensors and also a general lack of understanding of present capabilities. The offer, made throughout the world by NASA last year, of an opportunity to participate in its forthcoming series of Earth resources technology satellites has eliminated many of these reasons. NASA has undertaken to provide both a free platform, the ERT satellites, and free imagery on film and/or digital tape to all research workers who submit acceptable proposals. NASA will carry out digitization and geometric correction of the imagery at its ground data processing facility.

It was with the aim of stimulating an exchange of ideas on the subject of remote sensing, with particular reference to Earth resources satellites, that a small group of interested scientists and technologists met for an afternoon seminar in Imperial College, London, on October 22. Professor P. A. Sheppard (Imperial College), chairman of the Space Com-

THE long process of reinterpreting known geological regions in terms of the modern concepts of seafloor spreading and plate tectonics takes a further step forward with the publication of Oversby's analysis of the Tasman Geosyncline in next Monday's *Nature Physical Science*. This is not the first time that the concepts of the new global tectonics have been successfully applied to orogenic belts, for last year Bird and Dewey (*Bull. Geol. Soc. Amer.*, **81**, 1031; 1970) analysed the evolution of the Appalachian orogen in terms of lithospheric plate-continental margin tectonics and published a major article on the more general question of mountain building (*J. Geophys. Res.*, **75**, 2625; 1970). Because mountain building has been a central and fundamentally insoluble problem in geology since the early days of the subject, the second article marked a significant victory for the new global tectonics and proof that it may be successfully applied in a more detailed form than the simple conceptual model.

Oversby's analysis—the first for the Tasman Geosyncline—also illustrates the applicability of the new global tectonics in detail—detail which cannot be summarized in a few paragraphs. In general terms, however, Oversby envisages the action of several tectonic cycles during the late Precambrian and Palaeozoic evolution of the southern part of the Tasman Geosyncline. For each cycle there was a time lag between the start of plate motion and its first

effects on the continental side of the trench complex of which the geosyncline now forms a part, and a similar lag between the cessation of motion and the final effects. On the other hand, the effects of each successive cycle probably overlapped those of the previous cycle temporally and geographically.

Other conclusions reached by Oversby are that the westward motion of the downwelling oceanic plate was probably slow and that the downwelling (consuming) plate margin seems to have migrated eastwards. The first conclusion is supported by the "relatively mild tectonism spread over a period of time, which does not seem to have produced very marked topographic relief" and the second by the eastward younging of plutonics. The absence of major topographic highs at any time would also explain the marked lack of gravity slide sheets in the Tasman Geosyncline and, combined with the slow eastward migration of the margin, could explain the predominant presence of steep structures—in short, the usual effects of plate interaction seem to have been mitigated.

In attempting to relate the known geology of the Tasman Geosyncline to plate tectonics, Oversby admits that his interpretation may not be universally accepted. His admitted aim, however, is to provoke discussion. In the sense that the concepts of plate tectonics will ultimately be accepted or rejected on the basis of their application or non-application in detailed situations, the discussion must be important.

The Tasman Geosyncline and Global Tectonics

poses. The chief areas seem to be in the location of large scale structures in sedimentary rocks which might form oil traps, detection of ore bodies in arid areas, in engineering geology and in detecting major regional fractures. In discussing possible applications, Dr D. E. Pedgley (Centre for Overseas Pest Research) described the need for regular coverage of potential breeding grounds for the desert locust in parts of the Middle East. The search is aimed at detecting areas of recent rainfall, where the females can lay their eggs. Finally, Dr W. T. Welford (Imperial College, London) gave a brief outline of the potential of an optical Fourier transform instrument for use on ERTS imagery. This presents particular problems because of the high density of scan lines in the return beam vidicon output (4,200 on 70 mm format).

ACETYLCHOLINE RECEPTORS

After Denervation

from our Neurochemistry Correspondent

A PROBLEM of interest to neurobiologists for some time has been that of the development of neural connexions. Many approaches, involving a variety of techniques, have been used in this rather diffuse field of study, but one of the more successful has been the observation of changes in neuromuscular synapses in developing animals, and in mature animals after the normal nerve supply to muscles has been interrupted.

Transmission at a mature vertebrate neuromuscular junction occurs by the release from nerve terminals, in response to the arrival of nerve impulses, of discrete quantities of a chemical mediator, acetylcholine (ACh), which interacts with specific receptors on the muscle surface, thereby causing a characteristic permeability change, which excites the muscle membrane. Sensitivity to ACh is normally restricted to small "end-plate" regions, where the nerve endings make contact with the muscle, but if a muscle is deprived of its nerve supply it becomes responsive to ACh along the entire length of its surface. Foetal muscle fibres are similarly uniformly sensitive to ACh, and only after birth is there a retraction of sensitivity to the limited area of contact with the nerve; this occurs in parallel with the development of the characteristic end-plate structure. Reinnervation of a denervated muscle in a mature animal can also effect a similar shrinkage of ACh sensitivity; it seems therefore that this selection process requires some sort of neural influence.

Localization of ACh sensitivity in intact muscles is not entirely precise—

some extra-junctional responses have been obtained, especially at the ends of fibres, where they fuse with tendons, but the sensitivity of ACh at these sites is considerably less than at the end-plate itself. Until comparatively recently it was assumed that the responses to ACh of both junctional and extra-junctional sites were more or less analogous, but in two articles in the current issue of the *Journal of Physiology* (218, 85 and 101; 1971), Anne Feltz and Alberto Mallart suggest that qualitative differences may exist between them.

Feltz and Mallart studied responses to ACh by iontophoretic application together with intracellular recording from muscle fibres. The characteristic end-

plate responses showed a high sensitivity to ACh, with a rapid rise time independent of the dose of ACh applied. By contrast, the sensitivity to ACh at extra-junctional sites was lower, and responses had a longer rise time, which increased with increasing doses of ACh.

Transmitter action at the neuromuscular junction normally causes a local fall in the membrane potential, which in turn triggers impulses in adjacent areas of membrane. By displacing the membrane potential artificially and measuring the resultant change in the size of the ACh-induced depolarization (end-plate potential or e.p.p.), it has been found that the null point for the e.p.p. is -10 to -20 mV;

Pulsars and Supernova Remnants

RECENT observations of the Vela pulsar (PSR 0833-45), which was the first pulsar seen to glitch, and CP 1919, the first pulsar to be identified, provide new clues to the nature and origin of these objects. In next Monday's *Nature Physical Science*, P. E. Reichley and G. S. Downs, of the Jet Propulsion Laboratory, CalTech, report a discontinuous change of period in the Vela pulsar between August 24 and September 4, 1971. The previous recorded glitch in this source took place between February 24 and March 3, 1969. The interval between glitches fits well with the planetary perturbation model proposed by C. Michel, in which the close approach of a planet in a highly eccentric orbit produces a tidal starquake. But other theories remain viable, and perhaps the most significant aspect of the discovery of glitches in this pulsar is that it necessitates the revision of age estimates upwards, making it more likely that the pulsar is indeed as old as the Vela X supernova remnant which surrounds it.

Also in next Monday's *Nature Physical Science* are two independent reports concerning the non-thermal shell source close to CP 1919 in the sky. This is the source reported last year by Caswell and Goss (*Astrophys. Lett.*, 7, 141; 1970). W. M. Goss and U. J. Schwarz have now carried out a further survey of this part of the sky using the Westerbork array. They have found that the source has a shell structure, with the pulsar lying at the edge of the shell. The shell source itself is rather weaker than a typical supernova remnant (SNR), but comparisons are somewhat misleading because there is no such thing as a typical SNR.

Superficially, there are severe problems in identifying the shell source as an SNR associated with the pulsar CP

1919. The age of the pulsar is estimated as 10^7 yr, that of the shell source as 5×10^4 yr. The pulsar is widely believed to be at a distance of no more than 650 pc from Earth, yet the shell source is, if an SNR, at roughly 7 kpc. But the theoretical model for the origin of pulsars in supernova explosions is so strong, and the chance of finding a pulsar and an SNR aligned on the sky is so small, that these problems might be resolved.

In the same issue of *Nature Physical Science*, M. R. Kundu and T. Velusamy agree with the suggestion of Goss and Schwarz that a runaway pulsar could plausibly have reached the edge of the associated SNR since its formation. The more detailed analysis of Goss and Schwarz also suggests solutions to the other difficulties. The pulsar could be at the distance implied by taking the shell source to be an SNR, if the electron density in the line of sight to it is unusually low. In this case, the age difference could be resolved if CP 1919 is the older member of a binary pair, and the SNR results from the explosion of its companion comparatively recently. This would also allow plenty of time for the separation of the two components since the supernova in which CP 1919 formed, and it is not surprising, on the highly directional rotating neutron star model, that the younger associated pulsar cannot be seen.

Even more attractive is the possibility that the shell source is at 250 to 650 pc. In this case, it would represent a new type of galactic object, much weaker than an SNR, created either from the recent birth of the pulsar with a relatively long period of ~ 1 s from a star only marginally suitable to evolve into a neutron star, or, perhaps, from a second event later in the history of a more typical pulsar.

beyond this level ACh causes a potential change in the reverse direction from the normal. The "reversal" potential for the action of a particular drug or transmitter depends on the changes in the ionic permeability of the membrane produced by the interaction of the drug with its receptors. These changes can be deduced from the effect of altered ionic media on the position of the null point; in the case of ACh acting at the end-plate, the permeability to sodium and potassium ions only is increased by the transmitter.

In their second article, Feltz and Mallart describe experiments in which they used similar techniques to determine whether the same permeability changes are produced by the action of ACh at the extra-junctional receptors of normal frog muscles and at those induced by denervation. The results showed that although responses at both these types of receptors are also mediated exclusively by changes in cationic permeability, they have a reversal potential which is substantially more negative (-40 to -50 mV) than that observed at the normal neuromuscular junction.

The most rational explanation for this is that, during junctional and non-junctional responses, the permeabilities to sodium and potassium are altered relative to each other in a different fashion, thus indicating a real difference in ionic selectivity between junctional and non-junctional receptors. This selectivity could be caused by an altered association outside the end-plate between receptors and ion channels which have been opened up during their activation ("ionophores"), or by a genuine difference in the receptors themselves. Pharmacological studies have indicated that the ACh receptors which are induced after denervation are less sensitive to curare (Beranek and Vyskocil, *J. Physiol.*, **188**, 53; 1967), and of interest also are the recent experiments of Miledi and Potter (*Nature*, **233**, 599; 1971) on the binding of α -bungarotoxin to muscles. These studies suggest that two different classes of ACh receptor may exist at the end-plate itself, one of which has a low affinity for curare, and might therefore be analogous to the "slow-rise-time", "more-negative-reversal-potential" receptors of Feltz and Mallart. The toxin also binds to receptors induced after denervation, and its use will, no doubt, make possible their isolation and characterization, as well as that of the junctional and extra-junctional receptors of normal muscle. In the meantime, however, the discovery of measurable differences in the responses of these classes of receptor must stimulate further study of the influences which determine their formation.

PLANT PHYSIOLOGY

Phloem Transport

from a Correspondent

It may seem surprising that after more than forty years of intensive study by some of the most able plant physiologists of the day, the mechanism whereby organic substances, notably sugars, are transported in the phloem of plants is still unknown. The reason why this problem is unsolved is not hard to find. Transport occurs through narrow pipes—the sieve tubes—which, unlike the xylem vessels through which water moves, are alive and very sensitive to disturbance which readily leads to loss of function. Moreover, normal transport occurs only when the sieve tubes are connected at their ends with living cells which supply and receive the transported materials and there are some indications that association with living cells along the conducting strand is also essential. With such a complex multi-cellular system it is very difficult to obtain experimental results which can be interpreted unequivocally. Phloem transport is therefore an ideal subject for lively discussion, as was shown at a meeting organized by the Physicochemical and Biophysical Panel of the Society of Chemical Industry held in London on October 18 under the chairmanship of Professor P. E. Weatherley.

The most widely supported mechanism is still the one advocated in 1930 by the German botanist, Münch, and now usually referred to as "pressure flow". According to this hypothesis, movement of sugar into a sieve tube at one end (the source) and its removal at the other (the "sink") causes, by osmosis, a difference in hydrostatic pressure between the two ends and leads to a mass flow of water and dissolved substances along the tube.

A major drawback to the pressure flow hypothesis, as was pointed out at the meeting by Dr D. C. Spanner (Bedford College, London), is that if there are obstacles in the sieve tube lumen which significantly increase its resistance to linear flow, the pressure gradient required to force liquid through the tube at the rate observed would be much greater than that which is likely to occur. Both light and electron microscopic evidence suggests that such obstructions in the form of protein fibrils ("P protein") occur both in the lumen of the sieve tube and in the pores of the perforated baffles (sieve plates) which occur at frequent intervals along its length. Unfortunately the evidence from microscopic studies does not unequivocally establish the condition of the sieve tube in the normal functional condition and proponents of the pressure flow hypothesis contend that the apparent blocking of sieve tube

lumen and sieve plate pores with P protein is an artefact arising from the release of pressure which occurs when the material is being prepared for microscopic observation.

Spanner rejects this argument (*Nature*, **232**, 157; 1971) and believes that the pressure flow mechanism is untenable because of the impossibly high pressure gradients which would be required to force liquid through partly obstructed tubes. He has suggested that mass flow through the sieve tubes is brought about by an electro-osmotic mechanism operating across the sieve plate. Thus the sieve plates have a vital role in boosting flow at intervals along the transport pathways instead of being a hindrance as they are to the pressure flow mechanism.

The electro-osmotic mechanism was criticized by Dr E. A. C. MacRobbie (University of Cambridge) on the grounds that the fluxes of potassium ions required to maintain the necessary electrical gradient across the sieve plate in the manner proposed by Spanner would be several orders of magnitude greater than those normally found in living cells, and would be beyond the energetic capability of the cells concerned. She put forward an alternative hypothesis similar to that recently advocated by a Cambridge colleague, F. B. P. Wooding. Wooding has proposed (*Phloem* (Oxford Biology Readers, No. 15); Oxford University Press, 1971) that longitudinal movement of solution in the sieve tubes may be brought about by rhythmic undulations of P protein fibrils. Contraction and relaxation of protein fibres have already been implicated in the circulation of cytoplasm in some algal cells and in fungal filaments. There is some evidence that P protein fibres can be induced to contract by ATP in the same way as muscle fibres and flagellae, but further biochemical studies of P protein fibres are required before much credence can be given to this hypothesis.

The contractile fibre mechanism has a good deal in common with the proposal of R. Thaine that transport in sieve tubes occurs through "transcellular strands" by peristaltic pumping caused by contraction and relaxation of protein constituents in the walls of these strands. Dr Thaine was unable to be present at the meeting but Dr D. Aikman (University of East Anglia) presented some calculations which indicate that this mechanism is energetically quite feasible. In spite of the recent observations of Jarvis and Thaine (*Nature New Biology*, **232**, 236; 1971) most plant physiologists remain sceptical about the reality of transcellular strands. Elucidation of the structure of functional sieve tubes is required before any one of the current hypotheses can be supported.

Origins and Evolution of Ornithischian Dinosaurs

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Recent discoveries in the red beds of the Upper Trias have shed light on the history of the ornithischian dinosaurs. It is suggested that these dinosaurs had a monophyletic origin among advanced pseudosuchian thecodonts. Development of their rather bird-like pelvis involved backwards rotation of the pubis and subsequent growth of a prepubis. A conservative stock of primitive-looking hypsilophodonts, which persisted from the late Trias through the Cretaceous, lay at the core of ornithischian phylogeny.

BOTH orders of dinosaurs, Saurischia and Ornithischia, came into existence during the Triassic period. The study of saurischian phylogeny is a fairly straightforward matter since there is a wealth of information on Triassic forms¹⁻³. Early ornithischian history, in contrast, is obscure. Before 1962 the genus *Geranosaurus*, which is represented by a single jaw⁴, was the only undisputed Triassic ornithischian on record, but since that date knowledge of the earliest ornithischian dinosaurs has increased considerably; Upper Triassic forms have been discovered in southern Africa⁵⁻⁸,

China⁹, Argentina¹⁰, Nova Scotia¹¹ and, possibly, in Europe (my work in progress). This accumulation of new evidence permits critical reappraisal of ornithischian origins and evolution.

Monophyletic Origin

Recent discoveries show that the ornithischian dinosaurs had an almost worldwide distribution as early as the Upper Trias. Such a geographic range implies that ornithischian history extends well back into the Triassic period (that is to say, there must have been some considerable episode of dispersal before the Upper Trias). Ewer¹² has reiterated Broom's suggestion¹³ that Lower Triassic pseudosuchians such as *Euparkeria* are close to the ancestors of the Ornithischia. Unfortunately, none of these pseudosuchian reptiles shows any obvious tendency towards the ornithischian state of organization. The pseudosuchians and the ornithischians would seem, in fact, to be quite irreconcilable in terms of pelvis and ankle structure (Figs. 2 and 3). In the pseudosuchian pelvis (Fig. 2A) the pubis is directed anteroventrally; in the typical ornithischian pelvis (Fig. 2C) this bone consists of two branches—one (prepubis) extends forwards and the other (postpubis) runs down and back against the ischium. At first glance it seems unlikely that the ornithischian pelvis could have been derived from the pseudosuchian type of pelvis, but the pelvic bones of *Fabrosaurus*, an ornithischian dinosaur from the African Trias, indicate how such derivation might have occurred. In *Fabrosaurus* (Fig. 2B) the rod-like postpubis is developed in typical ornithischian

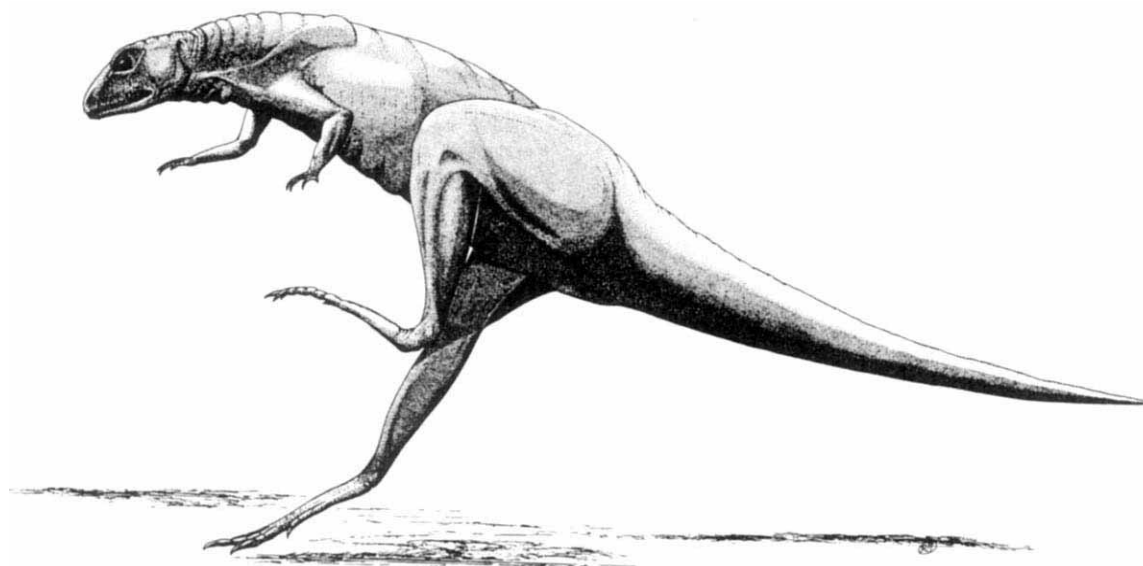


Fig. 1 Flesh restoration of the primitive ornithischian dinosaur *Fabrosaurus*, based on remains from the Upper Trias of southern Africa. This herbivorous dinosaur was about a yard long and was well adapted for fast bipedal running. Similar animals persisted until the close of the Cretaceous and probably represent the basal stock of the ornithischian dinosaurs.

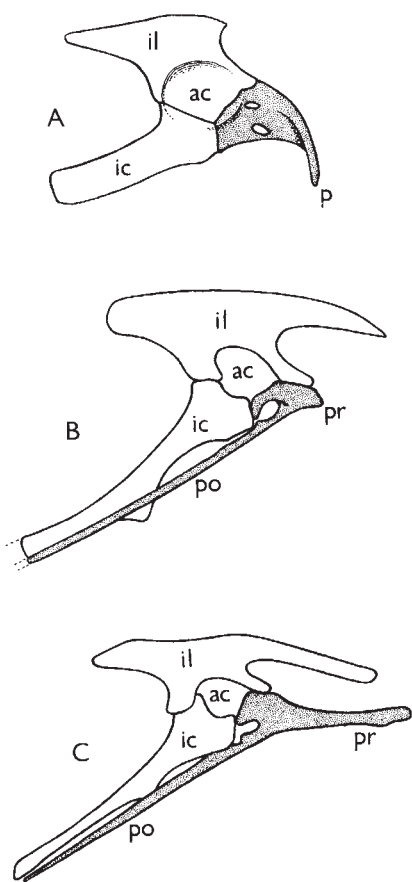


Fig. 2 Comparative diagrams of the pelvis in the pseudosuchian *Euparkeria* (A), the Triassic ornithischian *Fabrosaurus* (B) and the Cretaceous ornithischian *Thescelosaurus* (C). In each case the pelvis is viewed from the right side and the pubic bone is emphasized by shading. ac, Acetabulum; ic, ischium; il, ilium; p, pubis; po, postpubis; pr, prepubis. Diagrams A and C after Romer¹⁹.

fashion, but the incipient prepubis is merely a short stump of bone. This remarkable pelvic structure suggests that the ornithischian postpubis is homologous with the pubis in other reptile groups and that the prepubis is a neomorphic feature. Hence the pseudosuchian/ornithischian transition would have involved a backwards rotation of the pubis (to form the postpubis) and the subsequent development of a prepubis. Such a transition would also require a number of relatively minor changes, notably perforation of the acetabulum and extension of the ilium to form a salient anterior process. It may be concluded that the pseudosuchian and ornithischian types of pelvis are not totally irreconcilable in structure and that the latter may have arisen from the former.

But the origin of the ornithischian ankle joint still presents a formidable problem. In the ankle of *Euparkeria* (Fig. 3A), the astragalus is simply an extension of the tibia and the calcaneum forms a heel-like adjunct to the foot. In this rather complex tarsal system the "hinge" of the ankle separates the calcaneum from the astragalus. The ornithischian ankle (Fig. 3B) displays a somewhat simpler mesotarsal joint and the "hinge" of the ankle runs transversely beneath the astragalus and the calcaneum. The mesotarsal joint is in evidence as early as the Upper Trias (in the South American *Pisanosaurus*¹⁰) and cannot be envisaged as a simple modification of the pseudosuchian ankle joint. So it is clear that the ancestors of the Ornithischia must have differed from *Euparkeria* in ankle construction.

Although it may be deduced that the ornithischians arose from pseudosuchian reptiles which are at present unknown,

this still does not clarify the problem of ornithischian history during the Trias. The apparent hiatus between advanced pseudosuchians and early ornithischians arises chiefly from a deficient knowledge of terrestrial reptile faunas during the Middle Trias. There are, in consequence, no "intermediates" between the Lower Triassic pseudosuchians and the earliest known (Upper Triassic) ornithischians.

In spite of the obscurity surrounding early ornithischian history it is possible to infer, with a fair degree of certainty, that the ornithischian dinosaurs had a monophyletic origin and that the order Ornithischia is a "natural group". Such an inference is supported by several independent lines of reasoning. First, it is probable that the varied ornithischian types of the Jurassic and Cretaceous (iguanodonts, hadrosaurs, ceratopsians and the like) arose from ancestors within a single family, the Hypsilophodontidae. This basic assumption is sustained by the observation that advanced ornithischians (most particularly the iguanodonts and the hadrosaurs) may be derived from hypothetical hypsilophodont ancestors. This much, at least, of ornithischian history is fairly clear and has been adequately expressed by Romer¹⁴. Second, it transpires that all well known Triassic ornithischians are hypsilophodonts. These early dinosaurs display certain features (for example, toothed premaxillae, tibiae longer than femora) which are characteristic of the family Hypsilophodontidae. This evidence endorses the suggestion that the hypsilophodonts are at the core of ornithischian history. Finally, there is a remarkable homogeneity of structure throughout the order Ornithischia. Singular and diagnostic features, such as the biramous pubis and the prepubic bone (at the mandibular symphysis), are encountered in even the most aberrant ornithischians. It seems unlikely that structures as peculiar as these could have been developed in more than a single group of advanced pseudosuchians. All of these inferences point to a monophyletic origin for the order Ornithischia and emphasize the fundamental role of the hypsilophodonts in ornithischian phylogeny.

A Hypsilophodont Plexus

The earliest known ornithischians are therefore Upper Triassic representatives of the family Hypsilophodontidae. These Triassic hypsilophodonts may be divided, on the basis of cranial and dental characters, into two quite distinct groups. One group, exemplified by the African *Lycorhinus* (*Heterodontosaurus*)^{5,8}, may conveniently be termed the lycorhinids; these have, as one of their peculiarities, large canine-like teeth in both upper and lower jaws. The functional significance of these teeth is debatable, especially since the lycorhinids seem, like all other ornithischians, to have been herbivorous. It is possible that the canines were used as defensive weapons or that they expressed sex dimorphism, but there is no real evidence for either of these theories. *Geranosaurus*⁴, also from the African Trias, has

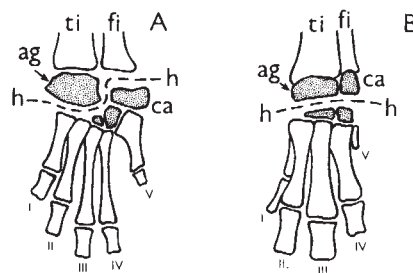


Fig. 3 Comparative diagrams of the right ankle (anterior view) in *Euparkeria* (A) and *Thescelosaurus* (B). In both cases the tarsal bones are shaded. ag, Astragalus; ca, calcaneum; fi, fibula; h, line of "hinge" through ankle; ti, tibia. Both diagrams after Romer¹⁹.

traces of an enlarged (canine?) tooth and might be regarded as an ally of *Lycorhinus*. Apart from this dental peculiarity the lycorhinids are further distinguished by having a deep longitudinal recess in the exterior of the maxilla and by the absence of teeth from the front of the premaxilla.

The other Triassic hypsilophodonts, best represented by the African genus *Fabrosaurus*^{6,7}, may be grouped together as the fabrosaur. The fabrosaur dentition lacks any canines; the premaxillary teeth extend to the tip of the snout and the lateral face of the maxilla is almost flat. These distinctions between the fabrosaur and lycorhinids (so striking that Romer¹⁴ accords the lycorhinids separate familial status) evidently reflect some evolutionary dichotomy of the ornithischian dinosaurs before the Upper Trias. This remarkably early episode of ornithischian diversification had never been surmised. The lycorhinids seem to have been restricted to southern Africa, whereas the fabrosaur are found as far afield as China (*Tatisaurus*⁹) and South America (*Pisano-saurus*¹⁰). It is also significant that the lycorhinids seem to have no post-Triassic derivatives whereas the fabrosaur may be regarded as forerunners of the Jurassic and Cretaceous hypsilophodonts. Hence the fabrosaur represent the earliest known portion of a hypsilophodont stock which persisted until the end of the Cretaceous. The lycorhinids, in contrast, seem to represent an extremely early and rather specialized hypsilophodont divergence which failed to survive the close of the Triassic period.

There is a sporadic record of primitive-looking hypsilophodonts, apparently derived from the Triassic fabrosaur, throughout the Jurassic and the Cretaceous. Hypsilophodonts are rare in the Lower Jurassic (Newman mentions¹⁵ an unnamed example from the English Lias) and unknown in the Middle Jurassic. The Upper Jurassic record is very much better; hypsilophodonts occur in the Morrison formation of North America (*Laosaurus*, *Nanosaurus*) and in the English Purbeck (*Echinodon*). Furthermore, Kühne has discovered hypsilophodont teeth, rather like those of *Echinodon*, in the Kimmeridgian of Portugal¹⁶. Apart from *Laosaurus* (which may just cross the Jurassic/Cretaceous boundary) only three hypsilophodonts have been found in the Cretaceous—one (*Hypsilophodon*) in the English Wealden and two (*Thescelosaurus*, *Parksosaurus*) in the Upper Cretaceous of North America. This succession of hypsilophodont dinosaurs, extending from the Upper Trias through the Cretaceous, might best be envisaged as an evolutionary plexus in which each genus represents an individual strand. This concept of a plexus (rather than a simple lineage) lacks the implication that any one genus might be directly related to another. This hypsilophodont plexus is fundamental to the whole of ornithischian phylogeny; it represents the ancestry, ultimately at least, of such groups as the iguanodonts, hadrosaurs and ceratopsians.

Ornithischian Phylogeny

It now remains to examine the relationships between the hypsilophodonts and other ornithischians. One of the earliest known ornithischians (apart from the Triassic forms) is *Scelidosaurus*, an armoured and quadrupedal dinosaur from the Lower Lias of Dorset. This genus has often been regarded as a stegosaur, but Romer has demonstrated¹¹ that it is, in fact, an extremely early ankylosaur. *Scelidosaurus* resembles other ankylosaurs (or "armoured dinosaurs") in lacking any salient prepubis. So it is natural to conclude that the prepubis remained undeveloped throughout ankylosaur history. This conclusion dismisses the general assumption¹⁴ that the ankylosaur pelvis had been derived from the typically ornithischian type of pelvis by reduction of the prepubis. *Scelidosaurus* probably shared a common ancestry with the hypsilophodonts of the Trias and so it becomes clear that the ankylosaurs, once thought to be an

exclusively Cretaceous group, have an immensely long history. There are no records of ankylosaurs from Middle and Upper Jurassic rocks. The Cretaceous ankylosaurs have not yet been studied in detail and it is, at present, impossible to discern any distinct evolutionary lines within the group. The ankylosaurs are often considered to have been terrestrial reptiles but some, such as *Edmontonia*, possess a solid secondary palate, a feature which is almost certainly indicative of amphibious habits.

Another group of ornithischians, the family Iguanodontidae, first appears in the Middle Jurassic and persists until the Upper Cretaceous. The iguanodonts are clearly derivatives of the hypsilophodonts; they differ from the hypsilophodonts in being larger and more complex in structure. But there is some evidence that the family Iguanodontidae did not have a single origin among the hypsilophodonts (that is to say, that several groups of ornithischians have, at various times, crossed the hypsilophodont/iguanodont boundary). In support of this hypothesis, there are several ornithischian dinosaurs which exhibit a peculiar mixture of hypsilophodont and iguanodont characters; for example, *Dysalotosaurus*, from the Upper Jurassic of East Africa¹⁷, which has sometimes been classified as a hypsilophodont, sometimes as an iguanodont. Since *Dysalotosaurus* has lost the more obvious hypsilophodont characters (the premaxilla is toothless and separates the maxilla from the nasal), it might best be regarded as an ornithischian which has just barely attained iguanodont status. A second example is furnished by the Upper Cretaceous *Thescelosaurus*¹⁸. This genus is invariably referred to the Hypsilophodontidae because it possesses premaxillary teeth and is very similar, in many respects, to the undoubted hypsilophodont *Parksosaurus*. But the hind limb of *Thescelosaurus* is typically iguanodont in appearance (that is, the femur is longer than the tibia). *Thescelosaurus* might therefore be considered as a hypsilophodont which shows a definite tendency towards the iguanodont grade of organization. The evidence from *Dysalotosaurus* and *Thescelosaurus* suggests that the family Iguanodontidae is a slightly artificial grouping and that it arose from the hypsilophodonts through iterative phases of evolution.

The origin of another ornithischian group, the suborder Stegosauria, presents even more of a problem. The armoured and quadrupedal stegosaurs (or "plated dinosaurs") appear in the Middle Jurassic and persist into the Lower Cretaceous. No Triassic or early Jurassic ornithischians are even vaguely suggestive of stegosaur ancestry. But the skull of *Stegosaurus* displays certain primitive characters; there is considerable contact between nasal and maxillary bones (in front of the orbit) and there is a broad and flat surface between the upper temporal fenestrae. Similar cranial features are evident in the Triassic hypsilophodont *Fabrosaurus* and in pseudosuchians such as *Euparkeria*. In most ornithischian dinosaurs there is no such nasal/maxilla contact and only a narrow zone separates the upper temporal openings. This evidence suggests that the stegosaurs were derived from the hypsilophodonts or (alternatively) that these two groups shared a common pre-Jurassic ancestry. It is unlikely that the stegosaurs could be related to either the ankylosaurs or the iguanodonts.

The origin of the suborder Ceratopsia (the "horned dinosaurs"), in contrast, is relatively easy to perceive. The Lower Cretaceous psittacosaur (family Psittacosauridae) are clearly hypsilophodont derivatives which are highly suggestive of ceratopsian ancestry. These psittacosaur resemble the hypsilophodonts in postcranial structure but show a slight advance towards the ceratopsian type of skull. Romer suggests¹⁹ that *Psittacosaurus* possessed a beak-like rostral bone, diagnostic of ceratopsians, in front of the premaxillae. This trend towards ceratopsian structure is carried even further in the Upper Cretaceous family Protoceratopsidae.

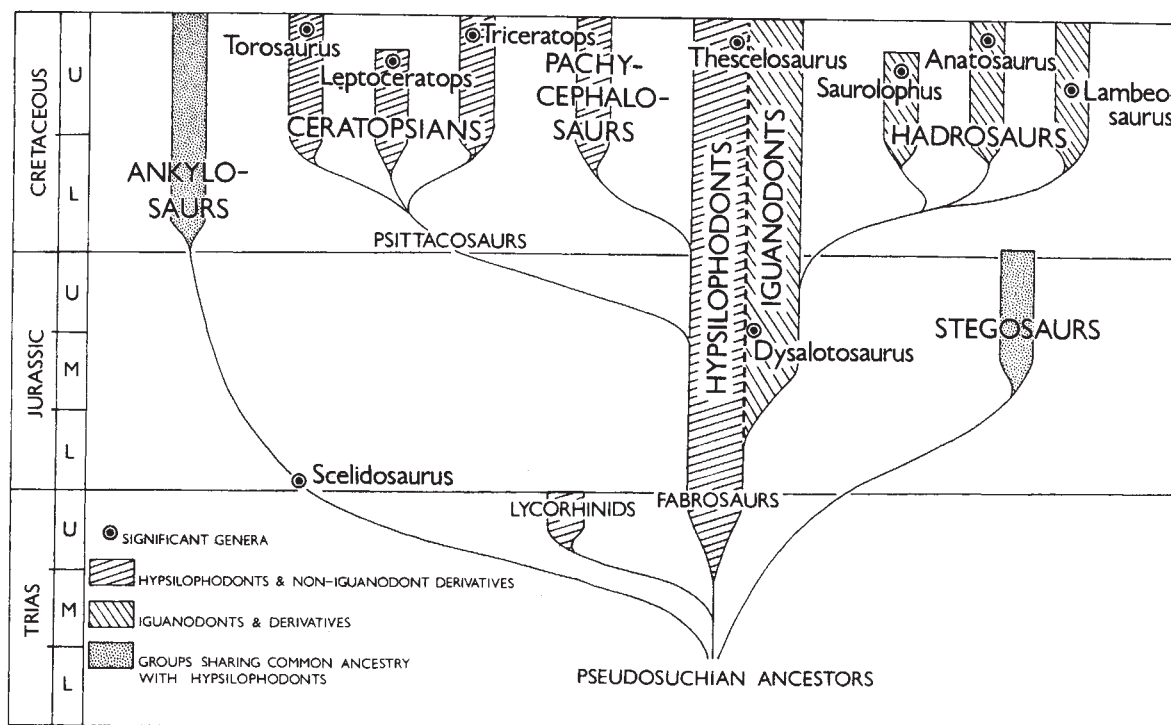


Fig. 4 Suggested phylogeny for the ornithischian dinosaurs.

The protoceratopsians are almost fully ceratopsian in appearance, but they still retain a few hypsilophodont characters; *Protoceratops*, for example, has teeth in the premaxilla. Even the true ceratopsians of the Upper Cretaceous—members of the family Ceratopsidae—display persistent traces of their hypsilophodont ancestry; *Triceratops*, perhaps the best known genus, has a primitive nasal/maxilla contact on the side of the snout. There can be little doubt therefore that the ceratopsians arose from the hypsilophodonts by way of psittacosaur and protoceratopsian stages. In a recent study of ceratopsian evolution Ostrom has discerned²⁰ three major lineages. One rather conservative lineage, terminating in *Leptoceratops*, seems to have retained the type of skull structure seen in *Protoceratops*. The members of a second lineage, exemplified by *Triceratops*, have the rear part of the skull extended into a moderately long bony “frill”. The third ceratopsian trend, culminating in *Torosaurus*, involves extreme elongation of this cranial frill. Expansion of the frill is probably to be correlated with enlargement of the jaw muscles and with the development of a very powerful jaw action.

The Upper Cretaceous pachycephalosaurs (troödonts or “dome-headed dinosaurs”) are characterized by a massively thick and solid roof to the skull. Apart from this peculiarity they conform to the hypsilophodont pattern of structure and they may be regarded, quite simply, as a late Cretaceous divergence from the hypsilophodonts. Galton suggests²¹ that pachycephalosaurs used their armoured skulls as battering rams during competitive courtship display—rather like living goats and sheep.

Ostrom has concluded²² that the Upper Cretaceous hadrosaurs (or “duck-billed dinosaurs”) were derived from the iguanodonts by way of intermediates like the poorly known *Claosaurus*. Within the family Hadrosauridae, Ostrom recognizes at least three major lineages. The members of one lineage (for example, *Anatosaurus*) show relatively little advance on the cranial structure of their iguanodont ancestors. The other two lineages display the progressive development of hollow cranial “crests”. One lineage includes hadrosaurs where the cranial crest is formed by exten-

sion of the nasals and the premaxillae (for example, *Lambeosaurus*). The other lineage comprises hadrosaurs, such as *Saurolophus*, where the crest is formed exclusively of the nasal bones. Furthermore, Ostrom has dispelled the popular concept of the hadrosaurs as aquatic or amphibious reptiles and has suggested²³ that these dinosaurs were active terrestrial foragers. More recently Galton has attempted to demonstrate some cursorial adaptations among the hadrosaurs²⁴.

All of the preceding discussion may be condensed into a single diagram (Fig. 4). This pictorial analysis of ornithischian phylogeny will doubtless require modification as new evidence comes to light but it would seem, for the present, to be a convenient summary of significant information. Such a scheme does at least emphasize the fundamental role of the hypsilophodonts in ornithischian history.

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Worldwide Deposition of Long-lived Fission Products from Nuclear Explosions

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The worldwide deposition of long-lived fission products is considered against the history of nuclear explosions. The present rate of deposition is much smaller than at the time of maximum fallout. The total amount deposited is consistent with the estimated amounts injected into the atmosphere.

THE radioactive debris from a nuclear weapon consists of fission products, unexpended fissile material such as uranium-235 and plutonium-239, and activation products—the result of the capture of excess neutrons in the weapon material or in the environment of the explosion. This vaporized mixture is forced upwards into the atmosphere by the tremendous release of energy in the explosion. As the cloud rises and cools, the radioactive products condense to form particles of solid debris. It is this particulate material, together perhaps with gaseous tritium (from fusion reactions) and carbon-14 (from the neutron reaction with atmospheric nitrogen), that later constitutes fallout. Nuclear radiations are emitted as the radioactive atoms disintegrate. The lifetime of the many products varies from fractions of a second to many years so that the radioactivity of the mixture decays in a complex manner.

Fission products from nuclear explosions have been present in the atmosphere for more than twenty years. At first the explosions were comparatively small so that the nuclear debris was confined zonally, within the troposphere and roughly to the latitude of the explosion sites. The stratosphere was penetrated for the first time by a thermonuclear device exploded by the United States in 1952. Since then there have been many large explosions¹ so that the subsequent dispersal of the debris, controlled by the meteorology of the stratosphere, has been world wide.

Of the fission products, caesium-137 (half-life thirty years) and strontium-90 (twenty-eight years) have, in the long term, the greatest biological significance in potential hazard to human populations. Since 1954 the concentration of these fission products has been observed at Harwell in rain from a number of collecting stations. Samples of rainwater are collected for periods of three months at (currently) twenty-eight stations² between latitudes 70° N and 76° S, including eight stations in the United Kingdom. The details of the techniques of sampling and analysis have been described by Cambray *et al.*³

The worldwide deposition of long-lived fission products may be calculated by the method of Stewart *et al.*⁴ by integrating the distribution by latitude of the concentration in rain, weighted by the distribution by latitude of rainfall⁵. Because all the sampling stations are based on land it is necessary to

assume that the concentrations measured in rain and the amount of rainfall are representative also of the oceans in the same latitude zone. The record for strontium-90 obtained by this means by Cambray *et al.*² is given in Fig. 1. This shows the annual deposition in northern and southern hemispheres, the total deposition and the calendar of high yield nuclear explosions^{2,6-8}. The low yield of tropospheric explosions have been ignored. Conventionally, the uncertainty attributed to the plotted points is 20% or 0.1 megacuries (MCi), whichever is the larger.

Annual Deposition

The shapes of the deposition curves conform generally to the calendar of nuclear explosions. Thus the annual rate of deposition increased to a maximum in 1959, the year following the first phase, during which about 90 "megatons of fission yield" were produced¹. There was a rapid decrease in 1960, a quiescent year, before explosions started again late in 1961. This massive series, yielding another 100 Mton, ended in 1962, and was followed by a peak in deposition rate during 1963 and then a steady fall until 1967. In this year the third phase of nuclear weapon testing started, a period of occasional explosions. (100 Mton of fission yield ~10 MCi of strontium-90 and corresponding quantities of other fission products.)

In the first phase of (high yield) nuclear explosions, before 1959, the individual explosions were usually of moderate size and took place at or near ground level. Much of the debris, including surface material entrained in the updraught, would have been lodged in the lower stratosphere. From here descent into the troposphere was prompt and in accordance with the subsidence mechanism that occurs (in the northern

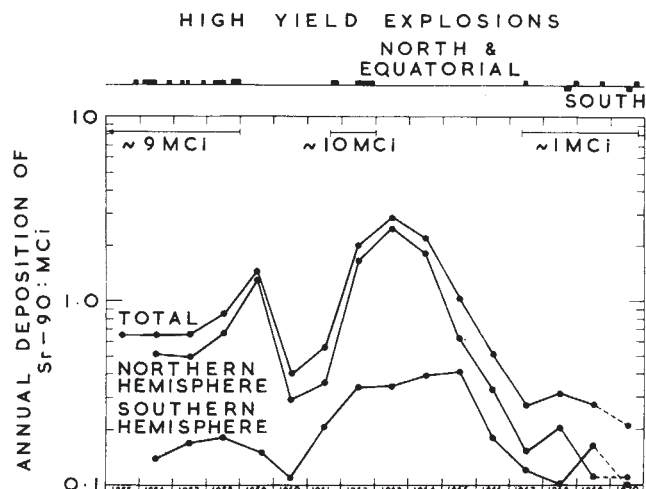


Fig. 1 Annual deposition of strontium-90.

hemisphere) in the early months of each year⁹. This explains the sharp decrease in deposition rate between 1959 and 1960. On the other hand, during the second phase 1961–62 the individual explosions were larger and generally above ground level. This implied insertion of debris higher in the atmosphere. The subsequent decrease in the total deposition rate—during the period 1964–67—was regular and more gradual, suggesting that the residence of the debris was controlled by a more general atmospheric mechanism, and not only by the sharp seasonal subsidence from the lower stratosphere. The characteristic rate of decrease was equivalent to a mean residence time in the stratosphere of sixteen months, or a half-residence time of eleven months. This estimate of residence time agrees with that obtained directly by Feely *et al.*¹⁰ from observations of the strontium-90 content of the stratosphere, sampled from aircraft and balloon.

During the period of regular reduction in the total annual deposition, the rate of deposition in the southern hemisphere increased in spite of the absence of high yield explosions during 1963–66. This has been explained^{11,12} by an exchange of debris between hemispheres, equivalent to a mean residence time against inter-hemispheric transfer of three to five years. The increase in the south was balanced by a decrease in the north at a rate faster than in the total deposition (Fig. 1). Therefore the residence time as observed at a northern station during this period would seem somewhat shorter than the real residence time against fallout.

The steady fall in total deposition rate continued until by 1967 the level was about one-tenth of the maximum in 1963. In 1967 the third phase of nuclear weapon testing started, yielding a few megatons during each of the years 1967–70. Because the total annual deposition has been approximately constant during this current phase the rate of injection of strontium-90 into the stratosphere was seen to be in fortuitous balance with the rate of depletion of the reservoir. This balance is also indicated by the high proportion, in debris observed at ground level during 1969 (ref. 8) and 1970 (ref. 2), from recent Chinese and French explosions.

The excess of strontium-90 deposited in the northern hemisphere over that in the southern hemisphere reflects the asymmetric injection of debris. The injections from the Russian sites at 52° N and 75° N have been largely contained within the hemisphere: the equatorial injections have been shared according to the latitude and season of the event⁹.

Throughout the whole period of observation the concentrations of long-lived fission products in surface air and rain, measured at a particular station, are related to the worldwide deposition. Thus comparison of strontium-90 deposited annually (MCi) in the northern hemisphere with the annual mean concentration (pCi/kg) in surface air at Chilton² (51½° N, 01° W) gives a ratio that lies, within a factor of two, about

10²⁰ kg. This ratio incorporates the latitude factor appropriate to Chilton and smoothes out the seasonal variation^{4,9,13}.

Cumulative Deposition

An alternative description of the history of the deposition of strontium-90 is given in Fig. 2, which shows the cumulative amounts corrected for radioactive decay. Although the annual deposition in the southern hemisphere has recently approached that in the northern hemisphere, the accumulated deposit in the north remains about four times that in the south. The pause between the first and second phases of nuclear testing is shown by the plateau in 1960–61 at about 4.5 MCi (total). This does not account for the whole of the strontium-90 produced up to that time: apart from radioactive decay and the residue in the stratospheric reservoir, some 3 MCi was deposited in the vicinity of the explosion sites¹. The second plateau, since 1966, represents an approximate balance between rate of deposition and radioactive decay of the strontium-90 already deposited.

The results obtained from these sampling stations² may be compared with the results from those operated by the United States Atomic Energy Commission¹⁴. The ratios of the worldwide deposition, estimated annually over the period 1958–69, have a mean (Harwell/USAEC) of 0.99 ± 0.10 s.d. The cumulative deposit of strontium-90 at the end of 1969 estimated in the present work is 12.42 MCi, compared with the American estimate¹⁴ of 12.25 MCi. In addition, the Harwell estimate of the cumulative deposit at the end of 1967 is 12.5 MCi: this compares with the value of 13 MCi derived by Hardy *et al.*¹⁵ from analyses of soil samples.

Because the extrapolation required from small samples to the global scale is so great that systematic errors might be expected to outweigh the random uncertainties, the agreement is surprisingly good. Each laboratory, using a separate sampling system and different methods of analysis and global integration, has achieved the same result, within experimental error. This result accords with the estimates¹ of injection, after making allowance for “close-in” fallout and for radioactive decay. Although it would be unreasonable to claim absolute accuracy, it is thus possible to account for the worldwide deposition of the long-lived fission products in a manner that is consistent, quantitatively, with the amounts that have been produced in nuclear explosions.

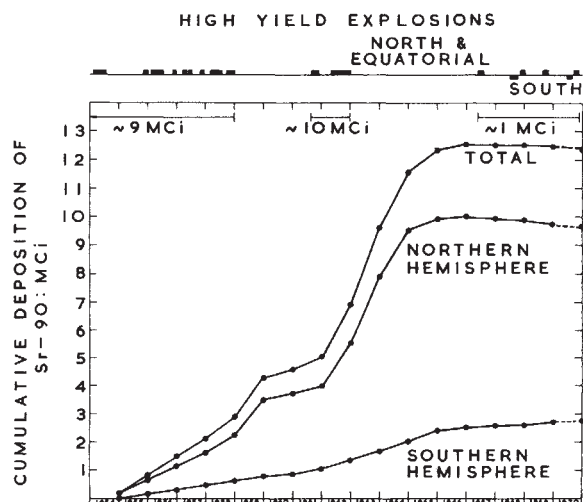


Fig. 2 Cumulative deposition of strontium-90.

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Light Chains from Fast and Slow Muscle Myosins

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Myosin from fast skeletal muscles consists of two large subunits (each about 200,000 daltons) and approximately four smaller subunits (in the range of 20,000 daltons). The light chains are heterogeneous in both size and charge, irrespective of the muscle type of origin. Moreover, the myosins from fast muscles of several species show a characteristic light chain pattern which is distinct from that of slower muscles.

THE bulk of the myosin molecule is composed of two large polypeptide chains each with a mass of about 200,000 g/mol¹⁻³, but some 10 to 15% of myosin seems to consist of low molecular weight material⁴⁻⁷. These "light chains" are thought to be involved in regulating the hydrolytic activity of myosin^{8,9}. A persistent problem associated with studies on the light chains has been the origin of their electrophoretic heterogeneity. The possibility exists that some of this heterogeneity may in fact arise from tightly bound contaminants or chemical modification of the proteins during their isolation. We shall describe a procedure whereby myosin from different species and types of muscle may be purified rapidly and the light chains compared in mass and charge.

By blocking the sulphhydryl residues of the light chains, it is possible to obtain a simple electrophoretic gel pattern with two to three bands whose relative proportions remain approximately the same with or without the presence of sodium dodecyl sulphate (SDS). Mobility in SDS gels permits an accurate determination of the mass of the components¹⁰⁻¹² while mobility in standard gels reflects the charge profile, so that the two electrophoretic techniques combined provide a sensitive "fingerprint" for comparing light chains from a variety of muscle tissues. Moreover, microdensitometer traces of 5 and 10% SDS gels can be used to estimate the number of moles of light chains in the myosin molecule.

Subunit Composition of Myosin

Polyacrylamide gel electrophoresis of rabbit skeletal (white) myosin in the presence of SDS shows predominantly three low molecular weight bands¹¹. Comparison of the electrophoretic mobilities with those of proteins of known molecular weight gives values of 25,000, 18,000 and 16,000 ± 5% for the three fastest migrating species (Fig. 1*a*). Lying between these components and the heavy chains (at the top of the gel) are minor bands in the 150,000 molecular weight range. This material can be almost entirely removed by ion exchange chromatography of myosin on DEAE-cellulose and probably represents a contaminant of myosin (Figs. 1*c* and 2). The remaining

electrophoretic bands are unaffected by all current purification procedures: exposure to 4.7 M NH₄Cl¹³ does not result in any selective removal of light chains (Fig. 1*b*), nor does fractionation with saturated (NH₄)₂SO₄ in the presence of 2.5 M LiCl affect the pattern (Fig. 4*a* and *b*)¹⁴. Light chains can, of course, be removed by treatment of myosin with alkali or guanidine hydrochloride or high concentrations of lithium salts, but these procedures inevitably lead to complete loss of ATPase activity. The only procedure described so far which

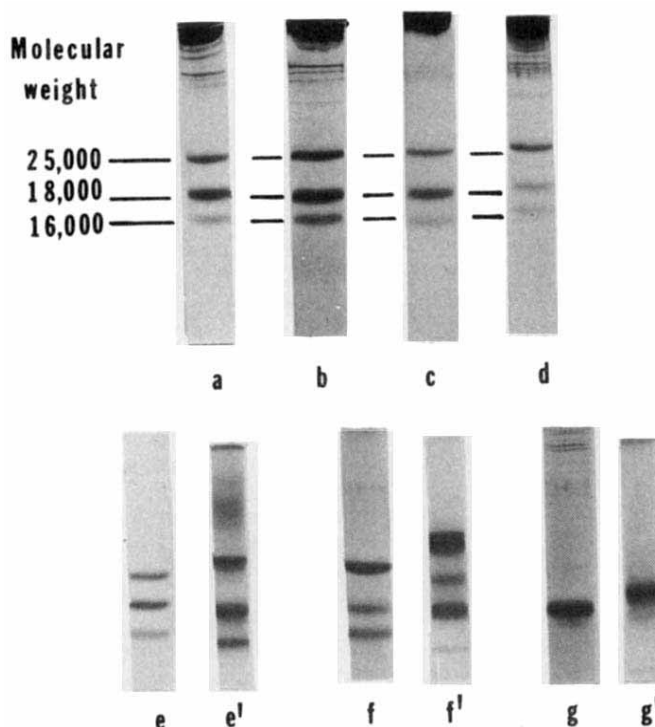


Fig. 1 Polyacrylamide gel electrophoresis of rabbit skeletal myosin and its light chains. (*e*', *f*', *g*') are standard 7% acrylamide gels (pH 8.5) prepared according to the Canalco manual. All others are 10% SDS acrylamide gels prepared according to Weber and Osborn¹¹. Myosin was routinely dialysed against 8 M urea, 0.01 M sodium phosphate, pH 7, containing 0.1% SDS and 0.1% β-mercaptoethanol. Molecular weights were determined from standard curves¹¹ and by running proteins of known molecular weight (chymotrypsinogen, β-lactoglobulin and myoglobin) together with the myosin. All isolated light chains shown here (*e*-*g*) were modified by performic acid oxidation (PAO) (for details, see ref. 17). *a*, Myosin prepared by conventional reprecipitation at low ionic strength. *b*, Myosin exposed to 4.7 M NH₄Cl for 10 min and precipitated with saturated (NH₄)₂SO₄ containing 4.7 M NH₄Cl, according to Gershman and Dreizen¹³. *c*, Myosin purified by DEAE-cellulose chromatography²⁵; see Fig. 2 for details. *d*, Myosin treated with 0.01 M DTNB, 0.05 M Tris, pH 8.5, 0.5 M KCl, 1 M urea (sometimes omitted) for 15 min^{3,15,17} and precipitated by ten-fold dilution. *e*, *e*', Total light chains from myosin. *f*, *f*', Light chains from DTNB-treated myosin (see *d*). *g*, *g*', Light chains from supernatant of DTNB reaction.

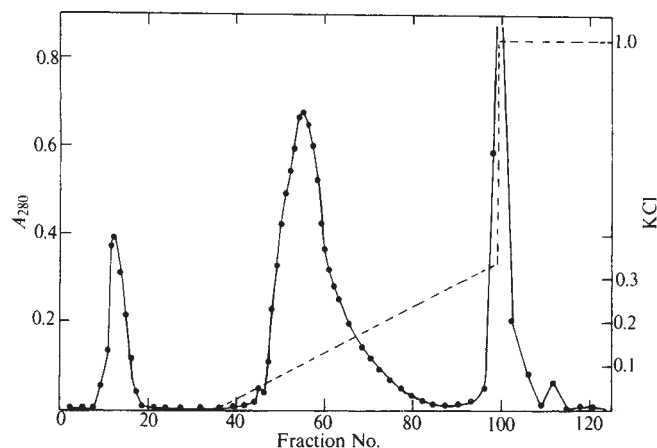


Fig. 2 Chromatographic purification of myosin. Minced muscle (in this case the anterior latissimus dorsi from chicken, 11 g) was extracted with 0.3 M KCl, 0.15 M phosphate, pH 6.5, 0.02 M EDTA, 5 mM $MgCl_2$, 3.3 mM ATP for about 30 min with stirring in the Sorvall blender. The extract was precipitated by a ten-fold dilution and taken up in 0.04 M $Na_4P_2O_7$, pH 7.5, against which it was dialysed. About 100 mg protein in 20 ml. was applied to a 30×1.5 cm DEAE (Whatman DE-52) column equilibrated with 0.02 M $Na_4P_2O_7$, pH 7.5. The protein was eluted with a linear gradient (0 to 0.5 M KCl in 0.02 M $Na_4P_2O_7$) of 400 ml. Fractions 47–71 were combined, dialysed exhaustively against 1% acetic acid and freeze-dried; the yield was 57 mg.

removes an appreciable portion of a light chain fraction without significantly altering the enzymatic activity is the reaction of myosin with 5,5'-dithiobis-(2 nitrobenzoic acid) (DTNB)^{3,15}. The light chains released by DTNB consist essentially of the 18,000 molecular weight species (Fig. 1*d, f* and *g*). (The same patterns could be obtained with myosin from chicken breast muscle.) After unblocking the DTNB-treated myosin with dithiothreitol, the Ca-ATPase, the EDTA-activated ATPase, and the actin-activated Mg-ATPase are about 70–80% of the control myosin. We were unable to find conditions in which all the light chains of 18,000 molecular weight dissociate. It required denaturation by alkali or guanidine hydrochloride to remove the remaining 18,000 molecular weight subunits as well as the 25,000 and 16,000 molecular weight components (Fig. 1*e* and *f*).

Most of the literature on light chains characterizes these subunits by some form of electrophoresis such as cellulose acetate strips⁹ or disc gel electrophoresis¹⁶ in standard buffers. The number of bands thus displayed has been variable and no clear picture of the composition of the light chains has emerged. The inclusion of SDS in the buffer system greatly simplifies the electrophoretic pattern; however, this procedure limits the characterization to one of size rather than charge. An equally simple pattern can be obtained in the absence of SDS if all the sulphhydryl residues of myosin are chemically blocked—preferably by performic acid oxidation¹⁷. The isolated light chains from the oxidized myosin show the same number of bands and approximately the same distribution of stain on the standard pH gels (Fig. 1*e'–f'*) as on the SDS gels (Fig. 1*e–f*). Thus there are predominantly three kinds of light chains in rabbit skeletal myosin—by the criterion of mass and charge. The 16,000 and 25,000 molecular weight subunits seem to be chemically related in sequence^{17–19}, whereas the 18,000 molecular weight subunit, or DTNB-light chain, is chemically quite distinct^{15,17} (see following article²⁰). As shown later, this light chain pattern will vary somewhat depending on the type of muscle from which the myosin is derived.

Stoichiometry of Subunits

Physical chemical methods for determining the moles of light chains in myosin cannot be used to discriminate among

the three different classes of light chains in rabbit skeletal (white) myosin. Values derived from light scattering²¹, sedimentation velocity and sedimentation equilibrium measurements have ranged from 2 to 3 mol of total light chains per mole myosin^{2,13}. The application of the “radioisotope dilution” technique to the problem of the stoichiometry showed that myosin contained 2 mol of DTNB-light chains and 2 mol of alkali-dissociated light chains¹⁷. Although chemical methods have the drawback of requiring large amounts of highly purified individual light chains, they are undoubtedly the most reliable means for determining stoichiometry. For obtaining a first approximation of the moles of light chains on small samples of whole myosin, however, a quick and relatively simple procedure is to measure the relative concentrations of protein in the light and heavy chain bands on SDS polyacrylamide gels. Such an approach is hardly new; one of the best examples of its application has been the determination of the stoichiometry of the twenty or so proteins in the ribosome²².

Owing to the great disparity in size and amount of light chains relative to heavy chains, it was considered best to run the myosin simultaneously on both 5 and 10% acrylamide gels. A known volume of a stock myosin solution was layered on each gel, with a minimum of ten times the concentration of myosin on the 10% as on the 5% gels. Since one must ultimately relate the concentration of myosin on the 5% gels to that on the 10% gels, the accuracy of the final calculation of light chains will depend in part on how precisely all volumetric

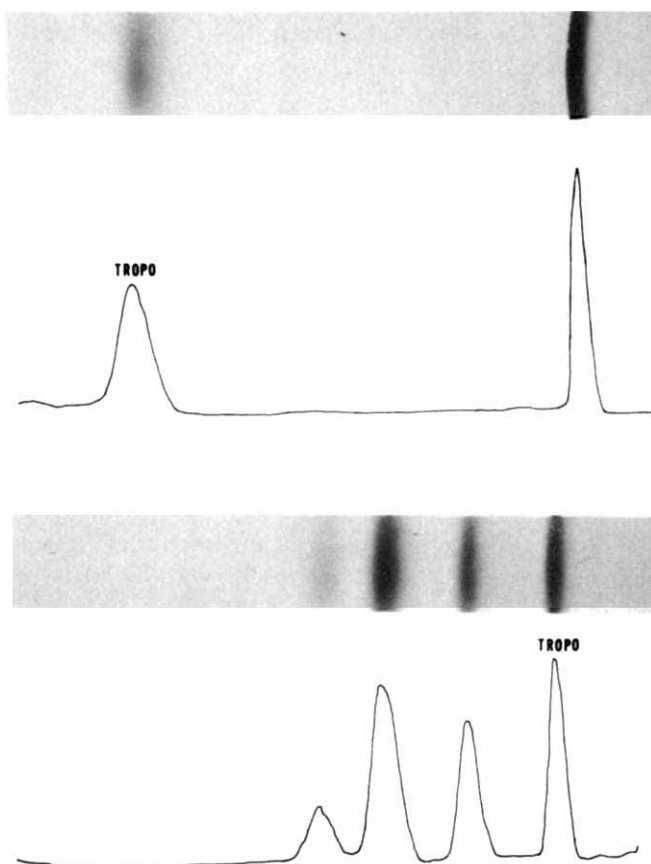


Fig. 3 Stoichiometry of light chains by densitometry of SDS gels. Samples of proteins were applied directly to the top of the gel with a 0.1 ml. Hamilton syringe. Gels were run about 5 h at 8 mA per tube, fixed overnight in 45% methanol, 9.2% acetic acid¹¹, and stained for 1 h in the same solvent containing 0.25% Coomassie brilliant blue or 0.5% aniline blue-black. Gels were destained electrophoretically and scanned on a Joyce-Loebl recording microdensitometer. The areas of the peaks (magnified about $\times 5$) were measured by planimetry and recorded as an average of 5 readings. Top, 5% SDS gel loaded with 100 μ l. of 0.1 mg/ml. tropomyosin and 100 μ l. of 0.1 mg/ml. myosin. Bottom, 10% SDS gel loaded with 100 μ l. of 0.1 mg/ml. tropomyosin and 150 μ l. of 2.4 mg/ml. myosin.

dilutions and applications of sample are made. Another potential source of difficulty is the different degree of staining of the 5 and 10% gels. To minimize this error, an equal amount of tropomyosin (molecular weight, 64,000) was applied to both gels, and any difference in area between the densitometer traces of the 5 and 10% gels was used to correct for differences in uptake of stain. A typical experiment is shown in Fig. 3. The heavy chains are unable to penetrate the 10% gels, but migrate well on the 5% gels. The light chains are well resolved on 10% gels but diffuse too much on 5% (or even 7%) gels to permit optimal microdensitometer traces. In using microdensitometry to determine protein concentration, it is imperative to demonstrate that the optical densities being measured are well within the linear range of the instrument. This was shown by running eight gels with increasing loads for each experiment. Plots of densitometer area versus load (or concentration) were reasonably linear and obeyed Beer's law. Results from several such determinations are summarized in Table 1. A striking feature of these data is the relatively constant value of total light chains for all these different myosins, despite variations in number and amounts of different light chains.

The validity of this approach depends largely on the extent to which the uptake of dye is affected by variations in the amino-acid composition of the subunits. Ideally, the degree of staining should depend only on protein concentration and be independent of the primary structure of the protein. Although the ideal situation is undoubtedly not realized, there are several reasons for believing the staining error to be small. (1) Our conclusions regarding the stoichiometry are in essential agreement with the chemical data on rabbit (white) myosin¹⁷. (2) The use of either Coomassie brilliant blue or aniline blue-black led to approximately the same results. (3) A study in which several dyes were used to estimate protein concentration reported a variation of about $\pm 10\%$ in absorbancy for a series of unrelated proteins of identical concentration²³. (4) Preparations of isolated light and heavy chains from rabbit skeletal myosin, the concentrations of which had been determined by Kjeldahl nitrogen, were run on 10% and 5% gels respectively and stained with aniline blue-black. The microdensitometer traces of the stained heavy and light chains were found to be identical for an equivalent amount of applied protein.

The results in Table 1 suggest that myosin from fast skeletal muscles contains approximately 4 mol of light chains: 2 mol of an 18,000 dalton subunit and a total of 2 mol of the 25,000 and 16,000 dalton subunits. Myosins from slow muscles seem to have equimolar amounts of a 27,000 and 20,000 dalton subunit. In the absence of more detailed chemical data on the separated light chains from cardiac and anterior latissimus dorsi myosin, the stoichiometry must be regarded as tentative.

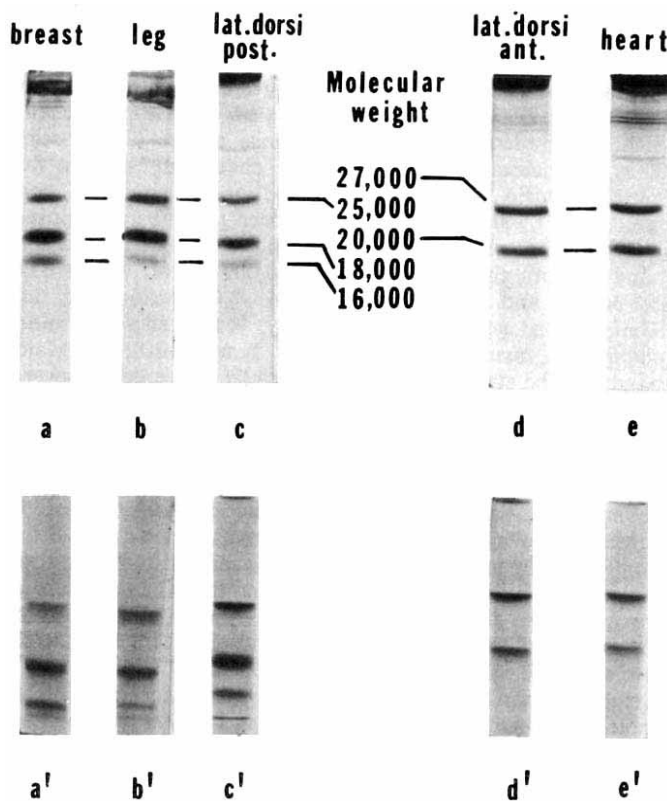


Fig. 4 Light chains from chicken muscles. All myosins were purified on the type column described in Fig. 2. Myosins from leg and breast muscles were in addition fractionated with $(\text{NH}_4)_2\text{SO}_4$ in the presence of 2.5 M LiCl¹⁴. *a* to *e* are 10% gels. *a'* to *e'* are PAO light chains prepared from the above myosins and run on 7% standard gels. Myosins were from: *a*, breast muscle; *b*, red leg muscles; *c*, the posterior latissimus dorsi muscle; *d*, the anterior latissimus dorsi muscle; *e*, cardiac muscle.

Comparative Studies

The light chains from various fast muscles show predominantly three classes of subunits either in SDS gels (Fig. 4*a*, *b* and *c* and Fig. 5*a* and *b*), or in standard acrylamide gels (Fig. 4*a'*, *b'* and *c'* and Fig. 5*a'* and *b'*). The pattern seems to be independent of species (in addition to chicken and rabbit myosins, sheep skeletal myosin shows the same three bands on SDS gels), and depends only on muscle type. The slower muscles, such as the anterior latissimus dorsi muscle or cardiac muscle, show two classes of light chains in the presence or absence of SDS (Fig. 4*d* and *e* and Fig. 5*c*). In addition to missing a 16,000 molecular weight subunit, the slow muscle

Table 1 Stoichiometry of Light Chains

Light chain	Rabbit leg and back		Rabbit white		Chicken breast		Chicken ant. l. dorsi		Chicken heart	
	%	(mol)	%	(mol)	%	(mol)	%	(mol)	%	(mol)
16,000	17	(0.8)	16	(0.7)	26	(1.2)	—	—	—	—
18,000	51	(2.2)	55	(2.0)	47	(1.9)	—	—	—	—
20,000	—	—	—	—	—	—	45	(2.0)	45	(1.6)
25,000	32	(1.0)	29	(0.8)	27	(0.8)	—	—	—	—
27,000	—	—	—	—	—	—	55	(1.8)	55	(1.5)
% total light chains in myosin	16.8 $\pm$ 1		14.1 $\pm$ 1		15.8 $\pm$ 2		19.3 $\pm$ 2		14.7 $\pm$ 1	

The value for the % of each light chain relative to the total light chain content (as measured in densitometer areas) represents an average of six to eight gels stained with both CBB and ABB. For "the percentage of total light chains in myosin" the contribution of the heavy chain needs to be determined; in this case, only ABB gels were measured, this dye giving more reproducible results than CBB. To determine the moles of light chain, a value of 470,000 daltons was assumed for all myosins. For example: moles of DTNB-light chain in rabbit white myosin = (14.1 %) (55%) (470,000)/(18,000) = 2.0.

myosins have light chains of slightly higher molecular weight than those in fast muscle myosin. The absolute values of the molecular weights are not as significant as the fact that mixtures of slow and fast muscle myosins show band splitting in the 25,000 and 20,000 region, with the slow muscle light chains higher in molecular weight than those of the fast muscles. Thus the heterogeneity of the light chains observed in fast muscle myosin cannot be explained on the basis of contamination by slow muscles, as has been previously proposed.

Red muscle myosin from rabbit interestingly shows a split band in both the 25,000 and the 20,000 region of the SDS gel pattern and a faint band in the 16,000 position (Fig. 5d). The standard gels are, paradoxically, too sensitive to minor changes in structure to show this band splitting. Major bands, in the standard gels, are generally bordered by several minor bands due to slight chemical modification of the side chains or aggregation or both. The SDS gels which are only sensitive to mass, however, suggest that the bands for the red muscle myosin represent a mixture of white myosin light chains and a pair of light chains similar in mass to those of cardiac or anterior latissimus dorsi myosins. To prove this point, two separate mixtures were run: one had white muscle myosin added to red muscle myosin, and the other had cardiac myosin added to red muscle myosin (Fig. 6). It is clear by inspection of the gels and their densitometer traces that the white myosin enhanced the 25,000, 18,000 and 16,000 molecular weight positions while the cardiac myosin enhanced the higher molecular weight bands in the 27,000 and 20,000 range. Thus it seems as if a so called pure red muscle may exist which has essentially two kinds of light chains.

This simple picture of a relationship between the light chain pattern and the speed of the muscle may, of course, be an over-simplification, based as it is on a limited study. However, for the present, it is tenable to hypothesize that the light chains

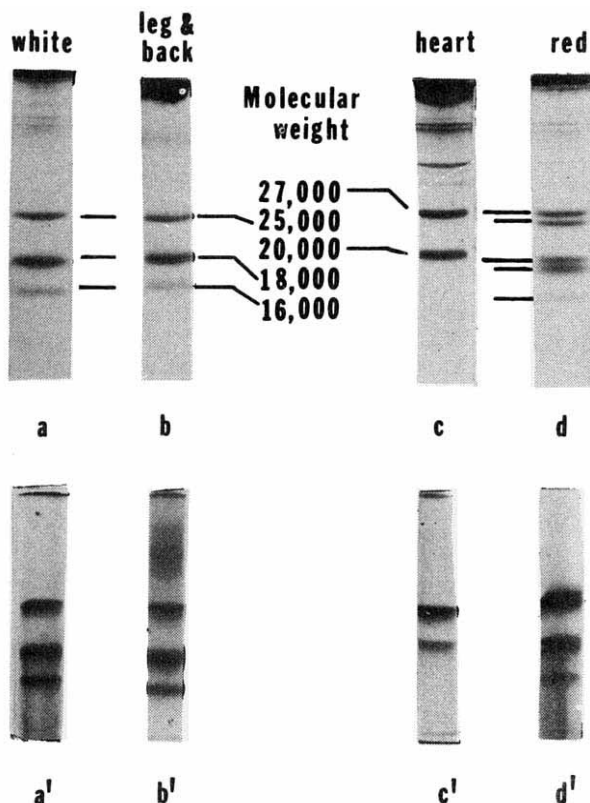


Fig. 5 Light chains from rabbit muscles. All myosins are column purified (see Fig. 2). *a* to *c* are 10% SDS gels; *d* is a 12.5% SDS gel. *a'* to *d'* are PAO light chains from the above myosins and run on 7% standard gels. Myosins were from: *a*, the white vastus lateralis muscle; *b*, the leg and back muscles; *c*, cardiac muscle—the additional strong band is actin; *d*, red muscles, including semitendinosus and crureus.

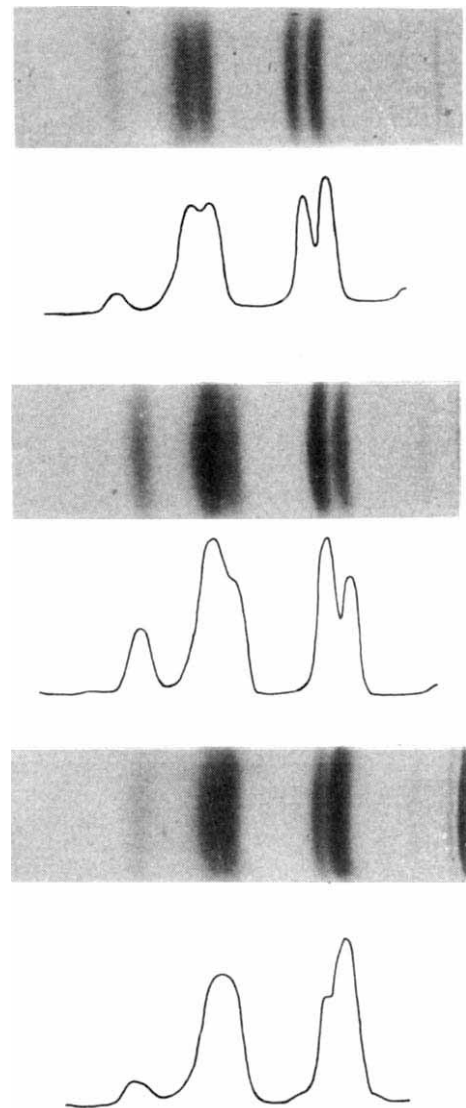


Fig. 6 Light chains from mixed muscles of the rabbit. All gels are 12.5% SDS gel; densitometer traces of these gels serve to emphasize the enhancement of particular bands. Top, red muscle myosin (Fig. 5d) showing two red bands in the 20,000 and 25,000 region and a faint band in the 16,000 region. Middle, red muscle myosin plus white muscle myosin showing an increase in density in the lower molecular weight bands. Bottom, red muscle myosin plus cardiac myosin showing an increase in density in the higher molecular weight bands.

may contribute to determining the response of the muscle by regulating the active sites of myosin^{8,9,24}.

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Chemical Studies on Light Chains from Cardiac and Skeletal Muscle Myosins

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Differences have been observed in the thiol sequences of light chains from cardiac and skeletal muscle myosins. Cardiac light chains from both bovine and sheep hearts contain the same thiol sequence (peptide A) which is present in the "alkali" light chains of rabbit and sheep skeletal myosin, but have two extra thiol sequences (peptides B and C). Chemical similarities have been demonstrated between myosin light chains from cardiac and slow skeletal muscle.

CHEMICAL studies of the light chains of rabbit skeletal muscle myosin have shown that these exist in two distinct classes as characterized by their thiol sequences^{1,2}, although electrophoresis on polyacrylamide gels and cellulose acetate indicates more extensive heterogeneity³⁻⁵. One of these light chains can be removed selectively from myosin by reaction with 5,5'-dithiobis-(2 nitrobenzoic acid) (DTNB)^{2,6}, in conditions where ATPase activity is little affected. This light chain gives a single band on polyacrylamide gels of molecular weight 18,000 and contains two thiol groups in identified sequences (peptides 1 and 2)². This we term the DTNB-light chain. The other chemical class of light chain is characterized by a single thiol sequence (peptide A), but gel electrophoresis shows the presence of two components of different molecular weights¹. These two light chains, together with any remaining DTNB-light chains, can be removed from myosin in various conditions, including alkaline pH⁷, acylation⁸, or treatment with denaturing agents such as urea or guanidine hydrochloride^{6,9}. We shall refer to these light chains not dissociated by the DTNB reaction as the "alkali" light chains¹. The stoichiometry of these two classes of light chains has been determined both by isotope dilution¹ and by densitometry of polyacrylamide gels¹⁰, showing that there are 2 mol of the DTNB-light chains and 2 mol of the "alkali" light chains per mol of myosin, consistent with its bipartite structure¹¹. Examination of the

active papain fragment of myosin, HMM S-1, shows that there is 1 mol of the "alkali" light chain in each HMM S-1, but the DTNB-light chain seems to be partially degraded during papain cleavage¹. If the light chains are dissociated from HMM S-1 or from myosin in mild conditions with 4 M lithium chloride, they can be reassociated with the heavy chains to recover enzymatic activity^{12,13}. Thus they seem to be involved in the hydrolytic activity of myosin.

Light chains of myosins from various animals give different mobilities on gel electrophoresis¹⁴, and SDS gels have confirmed differences in the molecular weights of light chains from fast skeletal muscle myosin and cardiac myosin (preceding article^{10,25} and our unpublished observations.) It was therefore important to examine the thiol sequences of cardiac light chains to establish whether they were related to the light chains from skeletal muscle myosin. Because of the limited size of rabbit hearts, cardiac myosin was prepared from beef and sheep hearts, and to ensure that differences might not be species specific, a comparative study was carried out with sheep skeletal muscle myosin.

The myosin was prepared from sheep leg muscle by standard methods¹, and from cardiac myosin as described before¹⁵. Treatment with DTNB was carried out as described previously^{1,6}, while the remaining light chains were dissociated from myosin by incubation in 4 M urea for 2 h at room temperature in the presence of 5 mM EDTA and 2.5 mM dithiothreitol at pH 8.0, followed by ten-fold dilution with ice cold water to precipitate the heavy chains. The light chains were recovered from the supernatant by freeze drying or by concentrating with a DEAE-cellulose column¹. Carboxymethylation with ¹⁴C-iodoacetic acid was carried out as described before¹. Gel electrophoresis was performed according to the Canalco-Chemical Formulation of Disc Electrophoresis (1965).

The carboxymethylated light chains were digested with α -chymotrypsin overnight at room temperature in 0.1 M ammonium acetate at pH 8.0 using an enzyme : substrate ratio of 1 : 80 (w/w) and a light chain concentration of 1-4 mg/ml. The digests were freeze dried, then dissolved in pH 6.5 pyridine acetate electrophoresis buffer diluted 1 : 1 with water, and applied to Whatman No. 3 MM paper at a loading of 1 mg/cm for electrophoresis at pH 6.5 (see ref. 20 for details of buffers). The radioactive peptides were further

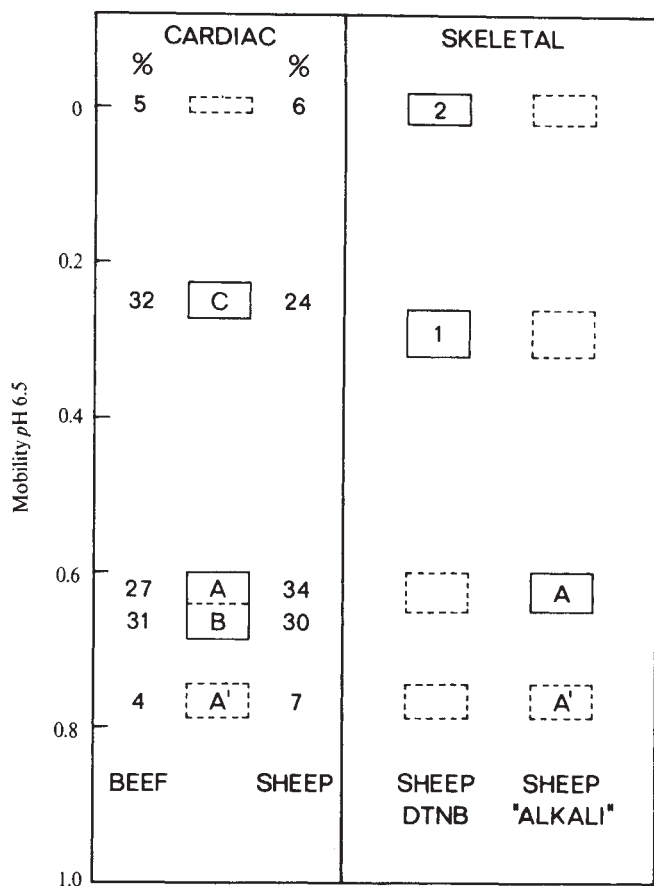


Fig. 1 Mobilities at pH 6.5 of radioactive CMCys peptides from chymotryptic digests of cardiac and skeletal myosin light chains expressed relative to aspartic acid = 1.0 (ref. 17). □, strong radioactive peptides; ▤, weak radioactive peptides. The percentage counts in each peptide were calculated for a single preparation of sheep cardiac light chains, and two preparations of the beef light chains. The nomenclature of the peptides is given in the boxes. (The mobilities of the peptides from sheep skeletal muscle were identical to those from rabbit muscle¹.)

purified by electrophoresis at pH 2 and strips containing them were exposed to performic acid vapour to produce the sulphone derivative of the carboxymethylcysteine; final purification was achieved by electrophoresis at pH 3.5. The peptides were eluted from the paper with pH 6.5 electrophoresis buffer diluted 1:4 with water. Amino-acid sequences were determined by the dansyl-Edman method¹⁶, and amide assignments made on the basis of pH 6.5 mobility changes after removal of aspartic or glutamic acid residues with the Edman reagent¹⁷.

Beef Cardiac Light Chains

Beef cardiac myosin was treated successively with DTNB and 4 M urea as described. Both supernatant fractions were reacted with ¹⁴C-iodoacetic acid and examined by gel electrophoresis. The light chains from the urea treatment gave two major bands, while those from the DTNB reaction showed the same two bands but in considerably reduced amounts. To confirm that these two fractions were similar, the proteins were digested with chymotrypsin and submitted to electrophoresis at pH 6.5. Autoradiographs showed the same three peptide bands for both the light chains dissociated by urea and those dissociated by DTNB. Thus the DTNB reaction removed a small amount of the total light chains, rather than a specific component as for rabbit skeletal muscle myosin. The amount of protein dissociated from cardiac myosin by DTNB was less than 1.5% of the myosin compared with 5–6% for rabbit skeletal myosin.

Thereafter, for larger scale preparations from beef cardiac myosin, the DTNB treatment was omitted and the total light chains were dissociated by urea incubation. Chymotryptic digests of the carboxymethylated derivatives were fractionated by electrophoresis at pH 6.5, giving three major and two minor radioactive peptide bands (Fig. 1). (Peptides A and B did not always separate, but with longer electrophoresis times two bands could be clearly discerned.) The percentage of counts in each band (Fig. 1) showed that the three major peptides occurred in roughly equal yield. The peptides were further purified by electrophoresis at pH 2, though the number of radioactive spots obtained at this pH was somewhat variable. The additional heterogeneity could be attributed to partial oxidation of the CMCys to CMCys sulphone which produces pairs of spots in the pH 2 dimension (Fig. 2). Such oxidation could be avoided by including thiodiglycol (0.5% v/v) in the wetting buffers, and this was necessary to facilitate separation of peptides A and B. Strips containing the radioactive peptides from the pH 2 electrophoresis were oxidized with performic acid vapour in a desiccator¹⁸ and final purification achieved by further electrophoresis at pH 3.5.

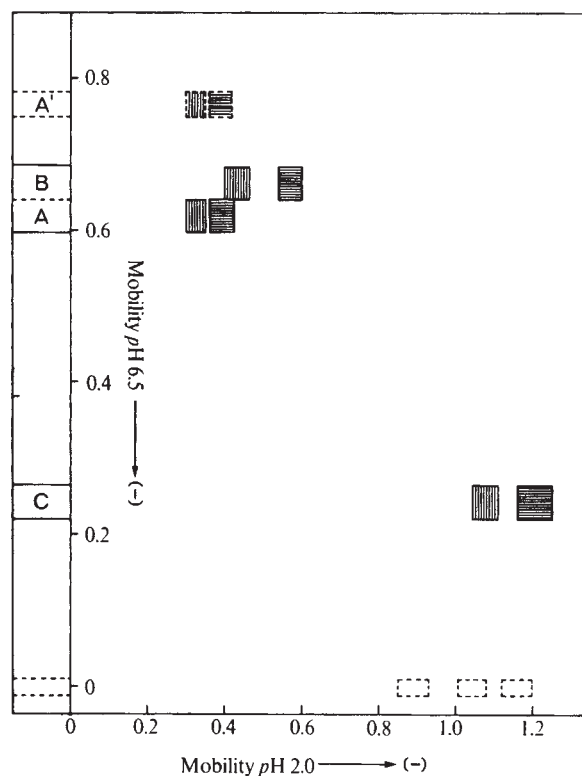


Fig. 2 Two dimensional peptide map of radioactive CMCys peptides from chymotryptic digests of beef cardiac myosin light chains. Mobilities at pH 6.5 expressed relative to aspartic acid = 1.0, and at pH 2 relative to serine = 1.0 (ref. 17). □, Strong radioactive peptides; ▤, weak radioactive peptides; ▤, unoxidized CMCys derivatives; ▤, oxidized CMCys sulphone derivatives. The nomenclature of the peptides is given in the pH 6.5 dimension.

The mobilities of peptides A and A' were the same in all electrophoretic conditions as peptide A and its deamidated derivative from rabbit "alkali" light chains^{1,2}, and the amino-acid compositions of both peptides were identical. Partial degradation by the dansyl-Edman method confirmed that the first six residues were the same as those determined for the skeletal peptide A, and from these data it was assumed that the sequences were identical (Table 1). The sequences of the two new peptides (B and C) were determined as shown in

Table 1. Amide assignments for peptide B were made as follows: after removal of Gly and Glu the mobility increased from 0.64 to 0.77 indicating that neutral residues had been eliminated; further degradation to give N-terminal Asp produced a non-radioactive tripeptide of mobility 0.52, confirming that the aspartic acid was charged. The mobility of peptide C at pH 6.5 showed that no amides were present. Thus the light chains of beef cardiac myosin contain three thiol sequences, one of which is identical to peptide A from skeletal "alkali" light chains.

The possibility remains that these three peptides do not derive from a single protein, since the light chains used in this experiment contained two components, of 27,000 and 20,000 daltons respectively¹⁰. Purification of a single component was achieved by chromatography of beef cardiac light chains before reaction with iodoacetic acid, on DEAE-cellulose (Whatman DE-52) in 50 mM tris-Cl at pH 8.0 with a gradient to 0.5 M KCl. Two peaks were obtained, the first of which gave a single major band on gel electrophoresis, while the second contained both proteins (Fig. 3 and ref. 19). Autoradiographs of digests of the two carboxymethylated fractions showed the same peptides in both fractions. The peptides were purified from the first fraction and amino-acid analysis confirmed the compositions of the three sequences (Table 1), showing that these three peptides derive from a single protein, whose mobility on gel electrophoresis corresponds to the 27,000 molecular weight light chain¹⁰. The presence of the same radioactive peptides in the second (mixed) light chain fraction may be accounted for in two ways: the counts may all arise from the contaminating 27,000 dalton protein or the smaller protein may contain some or all of the same thiol sequences. Measurement of the specific activities of the two fractions showed that the counts per mg in the first fraction were three times the value for the second fraction, which supports the hypothesis that the smaller protein contains fewer thiol groups. Further purification of the 20,000 dalton protein is essential before any sequence relationship can be investigated.

Sheep Cardiac Light Chains

Light chains were dissociated from sheep cardiac myosin with 4 M urea, and reacted with ¹⁴C-iodoacetic acid before digestion with chymotrypsin. The two dimensional electrophoretic separation of the peptides was similar to Fig. 2, and scintillation counting of the radioactive bands from the pH

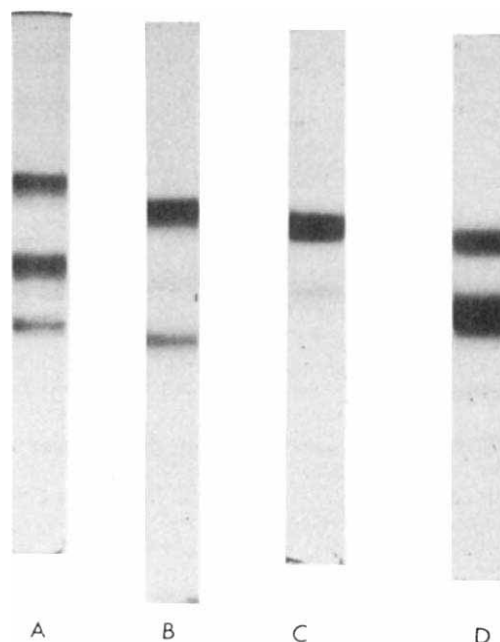


Fig. 3 Polyacrylamide gel electrophoresis of carboxymethylated light chains run on standard 7% acrylamide gels (pH 8.5) prepared according to the Canaco manual. A, Total light chains from chicken breast muscle myosin; B, "alkali" light chains from sheep leg muscle myosin; C, first peak fraction: beef cardiac myosin light chains; D, second peak fraction: beef cardiac myosin light chains.

6.5 electrophoretogram again showed approximately equal counts in each of the three major peptides (Fig. 1). The peptides were purified as before and amino-acid sequences determined (Table 1).

Thus both sheep and beef cardiac light chains are characterized by the same three thiol sequences, one of which seems to be identical to peptide A from rabbit "alkali" light chains¹, while the other two peptides do not resemble any of the known thiol sequences from rabbit myosin²⁰. In an attempt to show that rabbit cardiac light chains are similar in their thiol peptides to beef and sheep light chains, cardiac myosin was prepared from 3 rabbit hearts, and the light chains dissociated

Table 1 Amino-acid Sequences of Thiol Peptides from Cardiac Myosin Light Chains

Peptide A	
Rabbit skeletal A:	Met-Ala-Gly-Gln-Glu-Asp-Ser-Asn-Gly-CMC-Ile-Asn-Tyr
Beef cardiac A:	→ → → → → — — — — — — — —
Sheep cardiac A:	→ → → → — — — — — — — — — —
Peptide B	
Beef cardiac B:	Gly-Gln-CMC-Gly-Asp-Val-Leu
	→ → → → → → → →
Sheep cardiac B:	→ → → → → → → →
Peptide C	
Beef cardiac C:	Asp-Arg-Thr-Pro-Lys-CMC-Glu-Met-
	→ → → → → → → →
Sheep cardiac C:	→ — — — — — — —
	T2 T4 T1
	(→ →) (→ → →) (→ → →)
	[0.0] [-0.46] [1.05]
	T3
	(→ → → → —)
	[-0.33]

Amino-acid sequence data for chymotryptic peptides. —, Quantitative amino-acid analysis; →, sequences determined by the dansyl-Edman method¹⁶; T1, T2 and so on denote tryptic digestion products, with the mobilities at pH 6.5 given in brackets underneath the sequence.

Table 2 Amino-acid Sequences of Thiol Peptides from Skeletal Myosin Light Chains

DTNB-light chains	
Rabbit 1:	Thr-Thr-Gln-CMC-Asp-Arg-Phe
Sheep 1:	→ → → → →
Rabbit 2:	Lys-Asn-Ile-CMC-Tyr
Sheep 2:	→ → → → →
"Alkali" light chains	
Rabbit A:	Met-Ala-Gly-Gln-Glu-Asp-Ser-Asn-Gly-CMC-Ile-Asn-Tyr
Sheep A:	→ — — — — — — — — — —

Amino-acid sequence data for chymotryptic peptides. —, Quantitative amino-acid analysis; →, sequences determined by the dansyl-Edman method¹⁶; the amide assignments in this Table were determined for the rabbit light chain peptides².

and reacted with ¹⁴C-iodoacetic acid. Autoradiography of the two dimensional electrophoretic separation gave a pattern of spots similar to Fig. 2. The absence of species differences in the thiol sequences was confirmed by analysis of the thiol peptides from sheep skeletal muscle myosin.

Sheep Skeletal Light Chains

Both DTNB and "alkali" light chains were prepared from sheep leg muscle myosin, and chymotryptic digests of the carboxymethylated proteins fractionated by electrophoresis at pH 6.5. Autoradiographs showed that the DTNB-light chains contained two major peptides, while the "alkali" light chains gave a single major band (Fig. 1). Peptides 1 and 2 from the DTNB-light chains and peptides A and A' from the "alkali" light chains were purified and amino-acid compositions determined. These were identical to the equivalent peptides from rabbit light chains, but the sequences were determined for peptides 1 and 2 to confirm the identity (Table 2).

Table 3 Chemical Characteristics of Myosin Light Chains

Molecular weight *	Type of muscle	Thiol peptides †	Removal by DTNB
16,000	Skeletal	A	—
18,000	Skeletal	1, 2	+
20,000	Cardiac	—	—
25,000	Skeletal	A	—
27,000	Cardiac	A, B, C	—

* See preceding article¹⁰.

† See Tables 1 and 2 for the thiol sequences of peptides A, B, C and 1, 2.

Comparison of the thiol sequences of light chains from cardiac and skeletal muscle myosins shows that peptide A from the "alkali" light chains of skeletal myosin is conserved in cardiac myosin, but striking differences are also evident (Table 3). The DTNB-light chains are associated only with skeletal myosin. Reaction of cardiac myosin with DTNB removes only traces of light chain material, and the thiol peptides characteristic of the DTNB-light chains (peptides 1 and 2) are absent from cardiac myosin. Structural differences between the light chains of cardiac and skeletal myosins are indicated by the presence of two additional thiol groups (peptides B and C) as well as the higher molecular weight of the larger cardiac light chain. These structural differences may account in part for the variation in ATPase activity between cardiac and skeletal myosins, although evidence also exists for structural heterogeneity in the heavy chains¹⁵. The extent of sequence homology between the two cardiac light chains remains unclear.

Similarities between cardiac myosin and myosin from slow skeletal muscle have been demonstrated by ATPase inactivation

studies²¹ and gelelectrophoresis of the light chains^{10,25}. Chicken anterior latissimus dorsi myosin gave two light chain bands identical in molecular weight to those from cardiac myosin, while red muscle from rabbit appeared to be mixed in that all five molecular weight components were present¹⁰. A preliminary investigation has been carried out with cat myosins to analyse the thiol peptides from fast twitch and slow twitch muscles. Myosin was prepared from flexor digitorum longus (fast twitch) and soleus muscles (slow twitch) whose homogeneity had been tested by examination of the contractile response²². The isolated light chains were reacted with ¹⁴C-iodoacetic acid and chymotryptic digests prepared. Peptide purification showed that the fast muscle myosin contained both DTNB and "alkali" light chains, while the slow muscle myosin contained the three peptides characteristic of cardiac myosin (A. G. W., unpublished work). Thus the light chains of cardiac and slow-twitch muscles show similarities in their thiol sequences as well as in molecular weight. These results are consistent with the hypothesis that the light chains are involved in regulation of the contractile response^{12,13,23,24}.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Internal Constitution of Mars

THERE are three basic hypotheses to account for the low density of Mars compared with the Earth. Ramsay¹ and Bullen² suggest that both planets have the same overall chemical composition, but that differential self-compression in their respective gravitational fields combined with phase changes lead to differing densities. This hypothesis implies that the Earth's core represents a phase transformation from the non-metallic state of the mantle to a denser metallic state. Jeffreys³ and Urey⁴ assume that Mars and Earth consist chiefly of silicates and metallic iron. The composition of each of these phases is similar in both planets but Mars contains a smaller proportion of metallic iron. A third hypothesis has been proposed by Ringwood^{5,6}, in which the relative abundances of the common metals, Fe, Mg, Al, Si and so on, are similar in Mars, Earth and Venus, and also similar to abundances in the Sun and in Type 1 carbonaceous chondrites. Differences in density between the planets are caused by differences in the amount of oxygen present, Mars being more oxidized than the Earth and Venus representing an intermediate state. Additional hypotheses on the constitution of Mars have been proposed⁷⁻⁹ but these are essentially combinations of the second and third hypotheses mentioned above, and will not be considered further.

It now seems extremely improbable that the Earth's core represents a high pressure phase transformation of mantle material¹⁰, and the first hypothesis may accordingly be dismissed. There are also difficulties associated with the second hypothesis. Urey has pointed out that the moment of inertia of Mars requires most of the metallic iron in Mars to be distributed throughout the mantle, with only a small proportion in a core. But MacDonald¹¹ has shown that if Mars contained the chondritic abundances of U, Th and K, extensive melting in the interior should have occurred over 4.5×10^9 yr, so that most or all of the iron should have segregated to form a core. A further difficulty is that the atmosphere of Mars contains a substantial amount of CO₂ and that water is also present. If the Martian mantle consists of a mixture of metallic iron and silicates, equilibrium considerations dictate that the volatiles which would be degassed into the atmosphere consist chiefly of CO and H₂ rather than CO₂ and H₂O (ref. 6). Moreover, the red colour of Mars suggests the presence of ferric iron which is not readily explained by the redox state implied by the second hypothesis⁶.

In view of these difficulties we investigated the third hypothesis which maintains that Mars is composed of chondritic material in a higher state of oxidation than is displayed by the Earth or ordinary chondrites.

This investigation has been made possible by the measurement of the mean radius of Mars by the Mariner experiments and by planetary radar leading to determinations of the mean density and coefficient of moment of inertia, and by advances in our understanding of the role of phase transformations, not only within the Earth, but also in related compositions relevant

to the Martian models¹². The phase transformation behaviour of most of the minerals which may exist in Mars has been investigated experimentally at pressures up to 200 kbar, which corresponds to a range of depths including about 80% of the entire mass of the planet.

Table 1 Simplified * Composition of Type 1 Carbonaceous Chondrites

Component	Weight %
SiO ₂	33.32
MgO	23.50
FeO	37.37
Al ₂ O ₃	2.41
CaO	2.30
Na ₂ O	1.10

* All volatiles and sulphur omitted. Components present in amounts smaller than 0.5% omitted. NiO (1.9%) has been added to FeO. Iron is calculated as FeO, except that cases with varying proportions of iron calculated as Fe₂O₃ are considered in Table 2.

Table 2 Calculated Low Pressure Mineral Assemblages and Transition Pressures* for Oxidized Primitive Model Composition (Table 1) as Function of Oxidation State

Model	Fe ³⁺ / Fe ²⁺ + Fe ³⁺ (atom %)	MgO/ MgO + FeO (mol. %)	Oliv- ine (wt %)	Pyrox- ene (wt %)	Mag- netite (wt %)	Trans. press. (kbar)	
						A	B
I	67	100	20	40	40	151	262
II	35	68	60	19	21	135	229
III	5	54	94	—†	2	124	223

* Phase transitions A and B occur at pressures appropriate to a temperature-depth distribution with a uniform distribution of U, Th, and K throughout Mars in chondritic abundances¹¹.

† Other minerals—4%.

Transition A		Density increase (%)
Assumed to occur discontinuously at same depth	Olivine→spinel structure	10
	Forsterite→β-Mg ₂ SiO ₄	8
	Pyroxene→garnet str.	10
Transition B		
Assumed to occur discontinuously at same depth	Spinel→Sr ₂ PbO ₄ str.	9
	Mg pyroxenes→ilmenite str.	18
	(Mg garnet→ilmenite str.)	8
	Ca pyroxenes→perovskite str.	22
	(Ca garnet→perovskite str.)	12
	Magnetite→calcium ferrite str.	11

We take as our starting composition that of the metallic oxide components of Type 1 carbonaceous chondrites (Table 1) and have considered models having three different oxidation states, which result in different mineralogical compositions (Table 2). Model I is the most oxidized, all the iron occurring as magnetite, while the silicates are iron-free. Model II is an intermediate case, having about half of the iron present as FeO in solid solution in olivines and pyroxenes, and the remaining iron present as magnetite. This model has an oxidation state similar to the Karoonda chondrite¹² and mineralogically is the most plausible of those considered. Model III

is the most reduced, containing a maximum of FeO and minimum of Fe₂O₃. In this model, calcium is present as the larnite molecule.

Each of these models would be expected to undergo a series of major phase transformations over the pressure range occurring within the Martian interior and because of the differing mineralogical compositions, the transition pressures and corresponding density changes differ for each model. Nevertheless, experimental phase transformation studies^{12,14} strongly indicate that the relevant transformations for the models would dominantly occur in two stages, as in the Earth¹⁵. The first stage (transition A) is due to the transformation of olivine to the spinel or β Mg₂SiO₄ structure and of pyroxene to the garnet structure. The second stage (transition B) consists of transformations of spinels and garnets to denser silicate polymorphs characterized by octahedral coordination of silicon and probably possessing Sr₂PbO₄, ilmenite, perovskite and related CaFe₂O₄ or CaMn₂O₄ structures^{12,16-18}. Although transitions A and B would each occupy a substantial depth interval, we have simplified the models by assuming them to occur discontinuously at two characteristic pressures. The error introduced into subsequent calculations by this simplification is trivial. Our best estimates of the characteristic pressures for transitions A and B (at 1,000° C) based on the cited references are given in Table 3 together with estimates of the mean temperature gradients (dP/dT) of these transitions.

Table 3 Properties* of Principal Mineral Assemblages (Model II)

Assemblage	ρ_0 (g cm ⁻³)	K_0 (kbar)	α_0	Characteristic transition pressure at 1,000° C (kbar)	dP dT (bar/° C)
Olivine, pyroxene magnetite	3.79	1,300	3.5×10^{-5}		
Transition A	—	—	—	105	30
Spinel garnet mag- netite	4.06	1,900	2.9×10^{-5}		
Transition B	—	—	—	200	20
Post-spinel† as- semblage	4.50	2,300	3.0×10^{-5}		
High pressure form of magnetite‡	5.82	2,200	3.0×10^{-5}	200	20

* ρ_0 =zero pressure density, K_0 =zero pressure bulk modulus. α_0 =zero pressure coefficient of thermal expansion at 1,000° C.

† Post-spinel assemblage consists of high pressure polymorphs with ilmenite, Sr₂PbO₄, perovskite and calcium ferrite type structures.

‡ High pressure form of magnetite believed to have structure related to that of calcium ferrite or calcium manganite^{1,12,16-18,21}.

As our models are based in the first instance on chondritic compositions, it is appropriate to use the chondritic U, Th and K abundances in estimating the internal temperature distribution within Mars. The relevant thermal models have been calculated by MacDonald¹¹. We have used a temperature distribution shown in his Fig. 19 ($\epsilon = 10$ cm⁻¹) in our calculations. For a wide range of initial assumptions about boundary conditions, there is little difference between MacDonald's models in the outer 800 km of Mars which accounts for most of the mass and moment of inertia. Thus our subsequent calculations are not sensitively dependent on thermal considerations, provided chondritic abundances are used. Using the chondritic temperature-depth relationship, the parameters of transitions A and B (Table 3) and pressure-depth relationships which are obtained subsequently, the depths at which transitions A and B occur within Mars according to models I, II and III are fixed. These are given in Table 2.

We now have three models giving the mineralogy as a function of depth and wish to determine the density and pressure distribution throughout Mars for each of these models. The principal physical properties required are the zero-pressure densities, bulk moduli and thermal expansions of each mineral assemblage. The zero-pressure density ρ_0 is the decisive

property and can be accurately estimated for each assemblage from the densities of individual minerals determined either directly or indirectly^{12,14,19-21} combined with the relative proportions of individual minerals dictated by the particular compositional model. The zero-pressure bulk modulus (K_0) can be obtained with adequate precision by a combination of direct measurements on minerals and isostructural analogue compounds or alternatively using the seismic equation of state^{12,16,19,21}. Results obtained from the use of both methods are in good agreement. The thermal expansion (α_0) coefficients used are based on direct measurements on minerals and related isostructural analogue compounds¹⁹. The values of ρ_0 , K_0 and α_0 estimated in this way for each of the low pressure, intermediate pressure and high pressure mineral assemblages for model II are given in Table 3. Corresponding physical properties for the other models have been estimated similarly.

The density and pressure distributions throughout Mars for each model were then calculated, following the methods applied to the Earth's mantle by Clark and Ringwood²².

The isothermal pressure-density relation is taken to be the second-order Birch-Murnaghan equation of state²³

$$P = 3K_0 \left[\left(\frac{\rho}{\rho_0} \right)^{7/3} - \left(\frac{\rho}{\rho_0} \right)^{5/3} \right]$$

Densities were further corrected for expansion from 0° C to the chosen chondritic temperature distribution. The thermal expansion coefficient was taken to be independent of temperature and to vary with pressure according to the relation^{22,23}

$$\alpha/\alpha_0 = 1 - \frac{4P}{K}$$

The calculation proceeds inwards from the surface in steps. The pressure-depth relation is calculated for the model under consideration by an iterative procedure, yielding the density-depth relation. Mass and moment of inertia are then found by quadrature. The mean density of Mars thus obtained for each model and the corresponding moment of inertia coefficient, I/MR^2 , are given in Table 4.

Table 4 Mars: Model Densities and Moment of Inertia Coefficients

Model	Mean density	$\frac{I}{MR^2}$
I	3.921	0.392
II	3.972	0.391
III	3.991	0.391
IIA*	3.942	0.377
IIB†	3.942	0.373
Mars—(observed)	3.942 ± 0.005	0.377 ± 0.004

* Model IIA has differentiated crust 195 km thick with density of 3.0 g cm⁻³.

† Model IIB has differentiated core of HPP magnetite with radius of 1,638 km.

The mean densities of all three models are in close agreement with the observed density of Mars. Exact agreement could readily be obtained for models II and III by minor alterations of these models well within the uncertainty limits, but model I appears to be too light, in spite of the fact that it agrees better with the observed density than do the other models. The increase in density at transition A must be greater than seems to be allowed by the mineralogy in order to raise the mean density to the observed figure.

The moment of inertia coefficients for all models are substantially higher than the observed value. The increase of density with depth in Mars is thus substantially greater on the average than in the models. In order to achieve compatibility, the models must be amended to incorporate the effects of internal differentiation. Two differentiated models were considered. In the first (model IIA) a crust of density 3.0 g cm⁻³ is assumed to have differentiated from the Martian mantle. In order to yield a moment of inertia coefficient of 0.377, the thickness of

this crust on models II and III must be about 200 km, and small compensating density changes throughout the mantle must be assumed. A differentiated crust of this thickness appears excessive, although the possibility cannot be entirely eliminated. The second differentiated model assumes that the magnetite has segregated to form a core, analogous to the metallic core of the Earth. In model IIB, the magnetite in model II is differentiated to form a core with a radius of 1,638 km representing 21% of the mass of Mars. The magnetite is assumed to be present as the high pressure polymorph^{12,16-18,21} (HPP). Differentiation of a magnetite core according to this model eliminates the region occupied by the "post-spinel" assemblage below discontinuity B of models I, II and III. The moment of inertia coefficient is 0.373 which is slightly smaller than the best coefficient (0.377), although within its estimated error. Obviously, the assumption of a slightly smaller degree of differentiation could produce an I/MR^2 of 0.377. There is insufficient magnetite in model III to produce a large enough core to provide the correct moment of inertia coefficient. Accordingly, this extreme model can also be dismissed.

We conclude that a differentiated oxidized chondritic model can indeed provide the observed density and moment of inertia of Mars (Fig. 1). There is a range of possible models within this category. If differentiation has proceeded sufficiently to produce a core, it seems likely that a crust may also be present. Thus the most probable model would represent a combination of models IIA and IIB. This would still leave some magnetite in the mantle. To avoid the implication that only partial segregation of magnetite into the core had occurred, a variant of model IIAB towards model III might be assumed. This would imply a somewhat lower $\text{Fe}^{3+}/\text{Fe}^{2+} + \text{Fe}^{3+}$ ratio than model IIA and, consequently, a correspondingly smaller total amount of magnetite.

Another model tested assumed that the HPP magnetite core of IIB was entirely replaced by a troilite core (necessarily molten for chondritic U, Th and K abundances). This model provided the correct moment of inertia, but its density was too low. Nevertheless, it appears likely that some troilite would be permitted in a cosmochemically realistic model. Thus, the Karoonda chondrite¹³ contains about four per cent of (Fe,Ni)S. This is permitted within the range of possible differentiated variants of model II. It is also likely that a small metallic iron core^{9,24} would be permitted within the framework set by our overall boundary conditions—density, moment of inertia, chondritic abundances and variable redox state. But the presence of an iron core is not readily reconciled with the inferred oxidized state of surface rocks and atmospheric composition.

It is interesting that neither of the extreme class I or III models was satisfactory, while a range of models derived from differentiated versions of model II were satisfactory. It happens that model II representing an intermediate oxidation state similar to that displayed by the chondrite "Karoonda"¹³ is also the most reasonable one from a chemical and mineralogical viewpoint.

We have concluded that if Mars has an oxidized chondritic composition, then necessarily it must be a highly differentiated body, most probably possessing both a large core dominantly of HPP magnetite and a crust. It is satisfying that this conclusion is concordant with thermal history considerations. If Mars has the chondritic abundances of U, Th and K, the radioactive heat generated over 4.5 billion yr inevitably would have caused extensive internal melting and differentiation¹¹. Moreover the comparative dearth of craters on the Hellas region²⁵ indicates the occurrence of some dynamic crater obliteration process, indicating an "active" planet and perhaps suggesting currently active magmatism and differentiation. Our model is also consistent with the absence of a magnetic field on Mars, because the presence of a large, electrically conducting and convecting liquid core is not presumed.

Our work shows that the properties of Mars are consistent with an overall oxidized chondritic composition, in which,

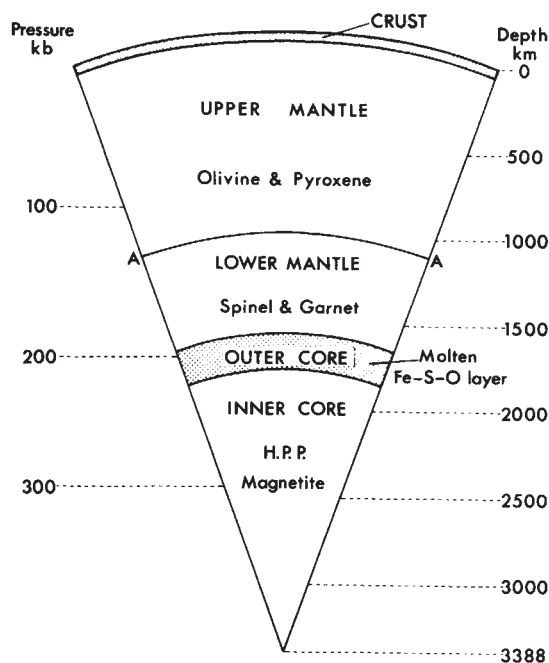


Fig. 1 Hypothetical section through Mars based on a variation of model IIB.

moreover, the relative abundances of metals are believed to be similar to those in the Sun. Self-consistent models of Earth and Venus can also be derived from chondritic or solar abundances, employing oxidation state as the major compositional variable^{5,6,12}. The postulate that mean oxidation states may vary between planets is inherently plausible. Chondritic meteorites themselves display a wide range of oxidation states. Important differences in oxidation states between lunar and terrestrial basalts and between their respective source regions have also been demonstrated. Finally, from a consideration of chemical processes occurring during the formation of planets, it is easy to understand how differences in mean oxidation states of planets might arise. Indeed, recent theories of planet formation^{6,26-28} which differ in important respects nevertheless have one aspect in common: they imply that it would be somewhat miraculous if all planets had acquired the same mean oxidation state.

Our hypothesis has the advantage that it is not necessary to make *ad hoc* postulates about the occurrence of metal-silicate fractionation in the solar nebula prior to planetary accretion by mechanisms which are poorly understood and for which no direct evidence exists. In contrast, the present redox hypothesis invokes a process which is known to have operated during planetary formation and which invokes relatively simple and well understood chemical equilibria.

In view of the imminent missions to investigate the surface composition of Mars it is worthwhile asking what predictions might be made on the basis of the two hypotheses discussed above. The redox hypothesis implies an overall chondritic composition for the planet. The K/U and Na/Ca ratios of surface igneous rocks would accordingly be expected to be much higher than occur, for example, in corresponding terrestrial and lunar rocks. On the alternative hypothesis that Mars consists of a mixture of metallic iron with silicates similar to those in the Earth's mantle, the K/U and Na/Ca ratios would be expected to be close to the terrestrial values. According to this latter hypothesis, metallic iron should be an important component of Martian rocks whereas on the redox hypothesis, metallic iron would be absent, and Martian rocks would be highly oxidized. The most important difference is that on the redox hypothesis strong differentiation of the planet would be expected, leading to a chemically and petrologically heterogeneous outer crust together with a dense core mainly of oxides. On the metal-silicate mixture hypothesis, internal heating by

K, U and Th has been insufficient to cause extensive differentiation, so that most of the iron has remained in the mantle.

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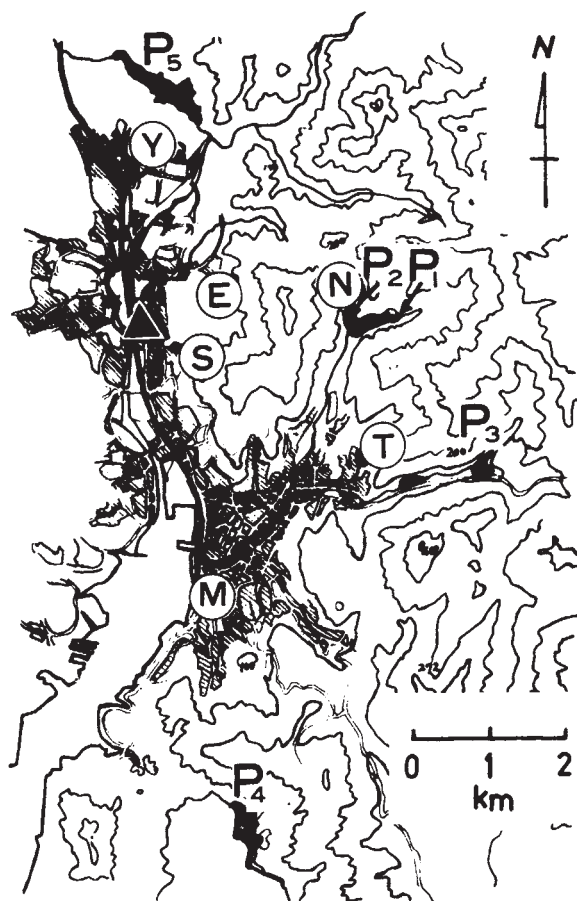


Fig. 1 Sampling locations of soils in Nagasaki. Δ , Centre of atomic bomb explosion; Y, Ieno-chō; E, Ehira-chō; S, Sakamoto-chō; M, Minamiyama-chō; N, Nishiyama-chō; T, Narutaki-chō.

Plutonium Content of Soil at Nagasaki

THE explosion of a plutonium atomic bomb over Nagasaki city in Japan took place at 1100 h on August 9, 1945. The bomb was detonated at a height of about 500 m above the place indicated in Fig. 1; the direction of the wind was from

the west. In July 1969, samples of surface soils (each 10 cm deep) were taken from the less disturbed surface of the ground, such as graveyards or grassland, so that the amount of plutonium remaining in the soil after 24 yr could be determined. The sampling locations are shown in Fig. 1; three different samples (N1, N2, N3) were taken from Nishiyama area where high radioactivities due to fission products had been detected after the explosion. The contour of the radioactivity dose rate in air in the autumn of 1945 is shown in Fig. 2.

The powdered dry soil samples (~10 to 50 g according to the plutonium content) were treated with 8 M nitric acid after adding ^{236}Pu tracer prepared by the reaction $^{237}\text{Np}(\gamma, n)^{236}\text{Np} \xrightarrow{\beta^-} ^{236}\text{Pu}$ (ref. 1). Ferric hydroxide co-precipitation was then induced from this sample solution in the presence of carbonate ion, followed by the dissolution in 8 M HNO_3 and solvent extraction with trioctylamine. This method had been

Table 1 Plutonium Contents of Soils from Nagasaki

Sample Location *	Sign	^{239}Pu (pCi kg ⁻¹)	^{239}Pu (mCi km ⁻²)	^{238}Pu (pCi kg ⁻¹)	$^{238}\text{Pu}/^{239}\text{Pu}$ (activity ratio)	^{90}Sr (pCi kg ⁻¹)
Ieno (1.6 km)	Y	22 ± 2	~1	7 ± 1	0.3 ± 0.05	324 ± 31
Ehira (1.1 km)	E	68 ± 4	~5	15 ± 2	0.2 ± 0.02	740 ± 40
Nishiyama (2.8 km)	N1	190 ± 8	~15	12 ± 1	0.06 ± 0.005	609 ± 39
	N2	473 ± 28	~38	43 ± 3	0.09 ± 0.009	794 ± 58
	N3	246 ± 12	~19	29 ± 3	0.11 ± 0.01	841 ± 55
Sakamoto (0.8 km)	S	76 ± 4	~6	20 ± 2	0.2 ± 0.02	968 ± 66
Narutaki (3.6 km)	T	46 ± 3	~4	(22 ± 2)	(0.4 ± 0.04)	988 ± 58
Minamiyama (4.4 km)	M	23 ± 2	~2	—	—	214 ± 22

* Distance from the centre of the atomic bomb explosion.

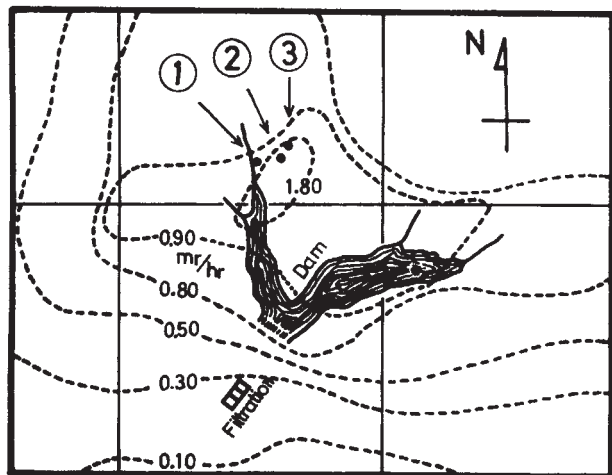


Fig. 2 Sampling points at Nishiyama area and the contour of radiation dose in the autumn of 1945.

proposed in our previous study² and some details were described in that article. The final determination of plutonium isotopes was made by α -ray spectrometry of an electrodeposited counting source, using a double gridded ionization chamber as detector. The α -spectrum from the sample (N3) of Nishiyama area is shown in Fig. 3 as an example.

Our determinations of the ^{239}Pu and ^{238}Pu contents are summarized in Table 1, together with the ^{90}Sr contents of these samples determined by the Japan Analytical Chemical Research Institute. It is remarkable that the ^{239}Pu contents at Nishiyama are still very high compared with the general level^{2,3} of this nuclide, even after 24 yr, although the contents of ^{90}Sr are not very different and are of the same order as the content of other soil samples (10 cm depth) in Japan². These facts may be due to differences in retention ability of the ground for various elements and are consistent with the experimental data⁴ obtained by radiotracer. The amount of ^{239}Pu per km^2 calculated from these data shows that the plutonium content of the sample N2 is about ten times higher than the plutonium content in other surface soil samples ($\sim 1\text{--}3 \text{ mCi/km}^2$) due to long-range fallout from subsequent nuclear test explosions^{2,3}. For the purposes of a discussion of the ratio $^{238}\text{Pu}/^{239}\text{Pu}$, it must be recalled that some ^{238}Pu was added recently during the burn-up in 1964 of the SNAP-9A satellite which used ^{238}Pu as an auxiliary power source.

These results can be compared with the data⁵ from the soils of New Mexico which were subjected to the plutonium atomic bomb test of July 16, 1945. In 1956, an activity of about 24 d.p.m. $^{239}\text{Pu} \text{ g}^{-1}$ soil was still found at a point 28 miles from the test centre.

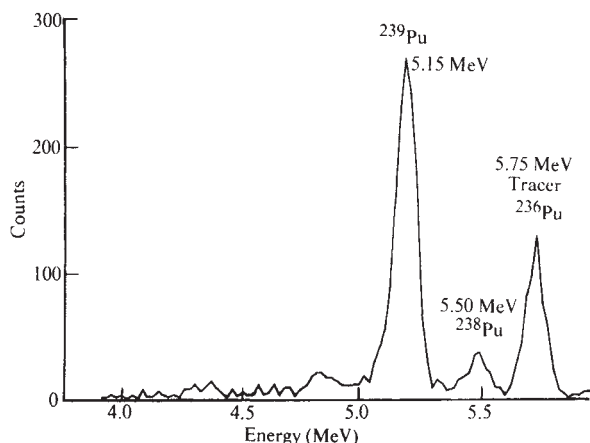


Fig. 3 Example of the α -spectrum of the plutonium fraction from sample N3. Sample weight, 10.2 g; chemical yield, 36%; measuring time, 1,450 min.

Further studies of plutonium remaining in Nagasaki have been made by the new method on the samples (P_1 to P_5) taken from the bottoms of four water reservoirs shown in Fig. 1 and have been reported elsewhere⁶.

These results may be important for the future studies of environmental radioactivity because they show that plutonium contaminants in soil are not easily removed naturally.

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Chrysotile Asbestos in Urban Air

THE industrial use of chrysotile asbestos is increasing and the question of whether its concentration in urban air constitutes a hazard has been raised. But measurements of asbestos in air near asbestos factories have proved negative with present analytical methods, so under the sponsorship of the Asbestosis Research Council we are developing a more sensitive technique. This article is a preliminary account of the estimation of chrysotile near a large asbestos textile factory at Rochdale, Lancashire. There are several uncertainties in the technique, so we were expecting to obtain only an order of magnitude estimate. Nevertheless this would have been an important figure to have because of the lack of data on the amount of asbestos in air. As it happened, we were only able to determine an upper limit for the chrysotile concentration which turned out to be three orders of magnitude lower than the threshold value for occupational exposure set by asbestos regulations. Obviously even more sensitive techniques are required and are now being developed.

We used an X-ray diffraction technique based on the measurement of the integrated area under the (002) peak of chrysotile. The equipment, which consisted of a Phillips 1010 generator, a vertical goniometer with a step scanning attachment, and a proportional counter with pulse height discrimination, could be reliably calibrated down to $10 \mu\text{g}$ of chrysotile compared with the 1 to 10 mg range reported by Crable¹, and was cross-checked by estimating the magnesium content of the calibration samples by atomic absorption spectroscopy. Sampling involved the collection of airborne solids from $1,000 \text{ m}^3$ (10^6 l.) of air by an electrostatic device (H. Litton Systems Inc.) in which up to $10,000 \text{ l. min}^{-1}$ are drawn through a 20 kV corona discharge. Particles in the air are electrostatically precipitated onto a plate and concentrated into $\sim 100 \text{ ml.}$ of liquid.

The collection efficiency depends on the size distribution of the particles and the sampling rate, but the size distribution of chrysotile in the atmosphere is not known. Therefore we estimated the collection efficiency indirectly by running the sampler in part of the asbestos factory where a low concentration of asbestos is known to occur (Fig. 1) and we found the collection efficiency to be almost 100% when the air is sampled at about $2,000 \text{ l. min}^{-1}$, dropping to between 25 and 50% at the rate of $10,000 \text{ l. min}^{-1}$, depending on the actual size distribution present. As we were aiming at only an order of magni-

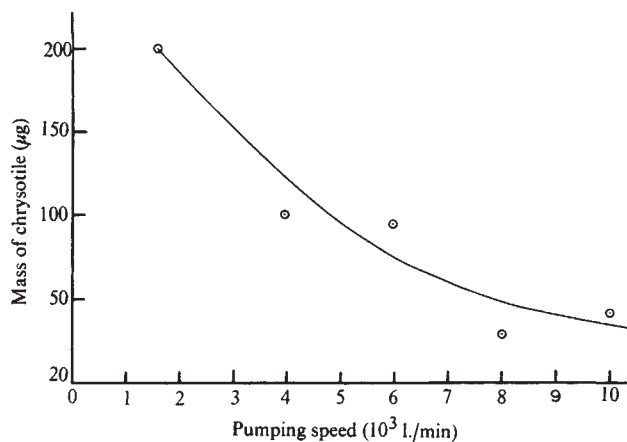


Fig. 1 Observed amounts of chrysotile in 5,000 l. of factory air, sampled at different rates.

tude assessment of asbestos in urban air, we were prepared to accept this uncertainty in the collection efficiency.

The map (Fig. 2) and Table 1 show the location of the sampling sites and the conditions in which the samples were obtained. The factory is in a hollow, and sampling site No. 2 is at the same height as the roof of the filter gallery, which is the chief air outlet from the factory. Sampling site No. 1 is about 30 foot higher than site No. 2. Sites 3 and 4 were in the gardens of houses, site 3 being about 5 km upwind of the factory and site 4 being about 300 m downwind.

All the diffraction traces (for example, Fig. 3) contained strong lines of kaolinite and quartz, probably from the local soil, which made the assessment of chrysotile difficult because the broad (001) line of kaolinite (7.18 Å) is close to the major (002) line of chrysotile (7.36 Å). Fortunately chrysotile is easily decomposed by boiling in 1 N hydrochloric acid whereas kaolinite is unaffected, so it should be possible to measure the amount of chrysotile present by subjecting the samples to acid leaching and measuring the corresponding reduction of the intensity of the composite X-ray band. The fact that this process led to no reductions in band intensity for any of the samples indicated that the amount of chrysotile present was below our detection limit.

We ought to have been able to detect 10 μg of chrysotile by itself, but clearly the presence of kaolinite may have reduced

Table 1 Weather Conditions during Sampling

Date (1970)	Site	Wind	Weather
April 22	1	SW moderate	Broken cloud
April 24	1	SW slight	Ground haze
April 27	1	NE moderate	Ground haze
April 29	1	SW moderate	Ground haze
May 6	1	S strong	Ground haze
May 13	1	N fresh	Ground haze
May 28	3	W light	Overcast, dull
May 28	3	W light	Overcast, dull
May 30	4	W light	Overcast, dull
May 30	4	W light	Overcast, dull
June 3	2	SW light	Overcast
June 10	2	SW slight	Heat haze
June 10	2	SW slight	Heat haze
October 23	1	W moderate	Broken cloud
October 23	1	W moderate	Broken cloud
October 23	1	W moderate	Broken cloud
October 28	1	N light	Broken cloud
October 28	1	N light	Broken cloud
October 28	1	N light	Broken cloud

the sensitivity. But the addition of 100 μg of chrysotile to our collected samples could easily be detected, so we can say that our samples collected from 1,000 m³ of air contained less than 100 μg of chrysotile—in other words, there was less than 0.1 μg of chrysotile per m³ of air. The threshold limit for occupational exposure set by the 1969 Asbestos Regulations² is 0.1 mg m⁻³.

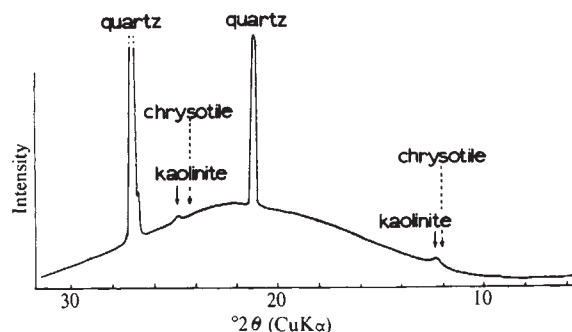


Fig. 3 X-ray diffraction pattern from a typical dust sample near the Rochdale factory.

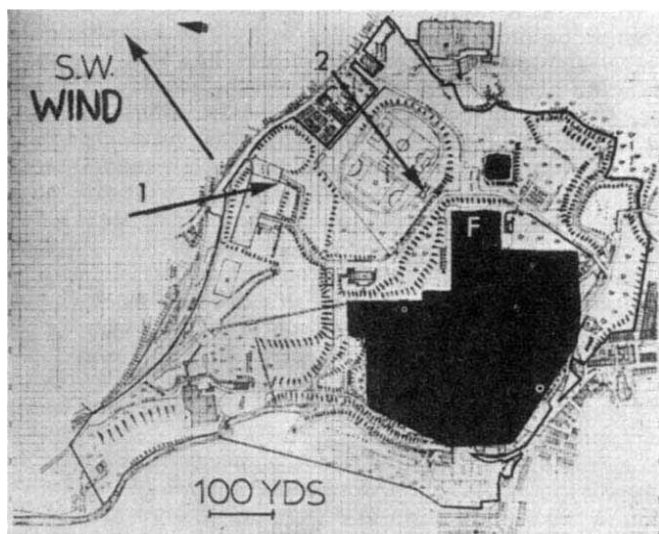


Fig. 2 Plan of T.B.A. factory, Rochdale. The sampling sites are indicated by the arrows 1 and 2. F, Position of the chief filter gallery exhausts.

A more sensitive method for estimating chrysotile is required, and we are developing a technique based on electron microscopy. Preliminary examinations under the electron microscope of samples collected by the Litton sampler indicate that the actual chrysotile level may be a further three orders of magnitude below the X-ray detection limit (that is, about 0.1 ng).

The samples have so far been collected in the close vicinity of the Rochdale factory. It is now proposed to sample air at certain representative urban and rural locations in UK and estimate their chrysotile content.

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² *Standards for Asbestos Dust Concentration for Use with the Asbestos Regulations 1969, Technical Data Note 13* (HM Factory Inspectorate, 1969).

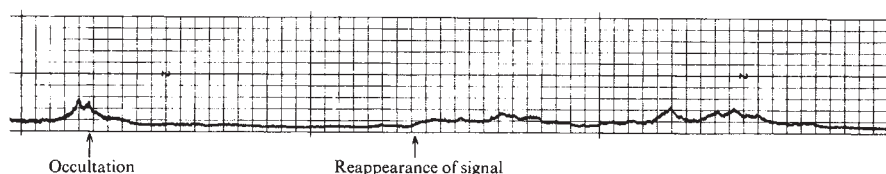
Post-occultation Reception of Lunar Ship Endeavour Radio Transmission

THE reception of radio signals from the orbiting lunar space-ship Endeavour after its occultation behind the lunar limb seems to confirm in part a previous suggestion¹. Similar observations arranged with the lunar command module during the Apollo 14 mission were unsuccessful because of interference from local emissions.

The transmitter normally used for communication between the lunar command module and the lunar landing party was turned on continuously during a period of lunar orbiting between 0026 and 0132 EST, August 3, 1971. The frequency of this transmission was 259.7 MHz with a steady square-wave modulation at 31.6 kHz. This transmission was received by a special receiver using the Air Force Cambridge Laboratory 150 foot radio telescope as an antenna. This radio telescope is located at Sagamore Hill near Hamilton, Massachusetts, USA (latitude 42°, 37', 55.6" N and longitude 70°, 49', 04.5" W).

The turning on of the spacecraft transmitter was delayed for several hours from the planned time because of astronaut activity required to ensure satisfactory air seals after the return of the lunar landing party. By the time turn-on was achieved, the Moon was so near to setting, as observed from Sagamore Hill, that unfortunately only one observation could be made.

Fig. 1 Record of receiver response (1 division = 5 s).



The interference encountered during the previous mission was not present during this observation, so that the receiver noise level was reduced from about 35 mV on the receiver output to about 2 mV. This improved sensitivity as compared with that of the Apollo 14 test allowed an excellent observation of the occultation of the spacecraft and the signal as the Endeavour transmitter moved behind the edge of the Moon. The signal reappeared 115 s later with an intensity many times the noise level (peaking at about 15 mV) and remained discernible for about 140 s. The reception is shown on the accompanying photograph of the pen-recorded strip chart (Fig. 1). Records were also taken by means of magnetic tape and digital sampling of receiver output voltage. The signal was further observed visually on an oscilloscope, and the characteristic modulation could be clearly seen both before and after occultation.

The height of the Endeavour's orbit above the lunar surface was about 108 km at the time of occultation and was reasonably uniform thereafter. Thus, the Moon ship passed along about 25° of its orbit as measured from the lunar centre during the period between the time that the radius vector from the ship to the lunar centre came perpendicular to the direction toward the Earth and the time of occultation. This represents about 960 km on the lunar surface directly below the Endeavour's orbit. During the 115 s that elapsed between occultation and reappearance of the signal, the ship passed over an additional 177 km of the lunar surface; then, during the renewed signal, the ship passed through 7° more of orbit, or a lunar surface distance of 215 km. Projected on the Earth's direction vector (which does not lie in the plane of orbit, because of the inclination necessary to ensure that the orbit would pass over the relatively northern landing site), this progress is measured by 175 km on the lunar surface.

The surface distance passed over between occultation and the return of the signal when projected on the line of signal is about 135 km. This distance places the Endeavour transmitter

63 km below the point where the Earth line of sight is tangent to the lunar surface. At the time when the signal finally ended, the "line of sight" from the Earth to the Endeavour passed about 158 km beyond the limb of the Moon. The librations of the Moon were all small at the time of these observations and the relatively insignificant corrections for these librations have been included in these figures.

Several possibilities for explaining these results may be considered. The passage of a refracted wave through the bulge of the Moon, as suggested for much longer wavelengths in previous articles^{1,2}, seems unlikely because the small refraction angles indicated would require much too small a refractive index for the lunar surface material. Also, the Moon is rather rough in terms of this short wavelength and the attenuation for such a high frequency is much too high, as indicated by measurements of returned lunar material³. The possible existence of a temporary ionosphere produced by gas emissions from the spacecraft appears to be unlikely because of the large amount of material required for the necessary deflexion of such high-frequency waves (259.7 MHz).

The most probable explanation seems to be one of two possibilities. First, the signal was received from a surface wave carried around the Moon, in a way similar to the propagation of waves in the broadcast band on the Earth's surface^{4,5}. This explanation appears possible because of the extreme

dryness of the Moon and the low electrical conductivity of lunar surface material, as compared with seawater and to soil containing considerable ground water on the Earth. Second, the signal was received from the prismatic refraction of mountain formations in this part of the Moon, where paths through lunar material may be relatively short and the materials may have properties differing somewhat from the average. Work is proceeding to determine, if possible, which is more likely. It is hoped that a repeat test during the Apollo 16 mission can be arranged and that observations can be made for several orbits of the command module. It is also expected that observations during future lunar missions will make possible more definitive data on the radio-frequency properties of the lunar surface, at much longer wavelengths, which will also be important tools in exploring the interior of the Moon.

We thank the Air Force Cambridge Research Laboratory for the use of their 150 foot radio telescope and Dr Jules Aarons and the operators for their assistance. We thank Dr Edward Lilley and Mr Hayes Penfield, of Harvard College Observatory, for the use of their receiver and for many discussions. We also should like to express our appreciation to NASA and the crew of Apollo 15 for their cooperation.

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Statistical Mechanics and the Critically Branched State

THIS communication deals with controversies concerning a standard reference case for equilibria in polymer science, namely the equilibrium distribution for a random f -functional polycondensation system. On the assumption that all functionalities are equally reactive and that intramolecular reaction does not occur, this distribution was derived by different probability arguments by Flory^{1,2}, Stockmayer³, Good⁴, Whittle⁵ and others:

$$w_x = \frac{(fx - x)! f}{(fx - 2x + 2)! (x - 1)!} (1 - \alpha)^{f \cdot x - 2x + 2} \alpha^{x-1} \quad (1)$$

where w_x is the weight fraction of x -mer and α the conversion. Recently, Masson *et al.*⁶ have claimed that Flory's derivation contains logical errors in the use of *a priori* probability and have denied that certain probabilistic operations used by Flory were meaningful. From an amended probability argument, they derived a new distribution which in the same notation takes the form

$$w_x = \frac{(fx - x)!}{(fx - 2x + 1)! (x - 1)!} \left(1 - \frac{\alpha f}{2(f-1)}\right)^{fx - 2x + 1} \left(\frac{\alpha f}{2(f-1)}\right)^{x-1} \left(1 - \frac{\alpha f}{2}\right) \quad (2)$$

Distributions (1) and (2) are both normalized and share the same number average $DP_n = \langle x^{-1} \rangle^{-1}$. Distribution (1), however, describes correctly the unique dimerization equilibrium for $f=1$, whereas distribution (2) obviously does not. Moreover, distributions (1) and (2) differ vitally as regards the weight average ($DP_w = \langle x \rangle$), which diverges for distribution (1) at

$$\alpha_c = 1/(f-1) \quad (3)$$

This divergence was interpreted by Flory as the statistical basis of gelation at the gel point α_c . The behaviour of matter in the critically branched state, that is, of systems near their gel point, has been rationalized on this basis. For instance, sol-gel analysis⁷ accounts for measurements on many different systems.

The distribution of Masson *et al.* has a mean (DP_w) which does not diverge until α attains the value $2/f$, where the DP_n of both distributions diverges also. This point is the limiting conversion possible for a strictly tree-like gel (that is, one free from rings) and Bruneau⁸ has also sought to identify this point with experimentally observed gel points, thus implicitly denying the validity of sol-gel analysis. Masson has adduced experimental results in support of distribution (2) from measurements of equilibrium monomer concentrations in ionic polysilicate melts.

There are two methods of settling the controversies unequivocally. The first method examines the mutually contradictory arguments of various authors, all of which are based on probability theory, and removes the erroneous arguments by the only procedure valid in deductive science. That is, an axiomatic basis is defined for probability theory, and it is then shown which statements of any author are inconsistent with the defined axioms. (The history of the axiomatics of probability theory has itself been rife with controversy. Some problems in probability theory have different solutions according to the set of axioms adopted.) Great care has been taken by mathematicians to define various possible axiomatic bases of probability theory which appeal to them. Not without reason, large initial sections of texts on probability theory (that of Renyi⁹, for example) are taken up by this subject. The controversies surrounding polycondensation and gelation, though not lacking in power of rhetorical exposition, are too far from such a standard of rigour or from an agreed axiomatic

basis for the first method of settling them to be applied with any confidence.

The second method, which is therefore preferable and is adopted here, derives the correct answer afresh from statistical mechanics without probability arguments. It is important to make clear the rationale of this method. Statistical mechanics is actually the relevant universal discipline for calculating equilibrium distribution of molecules. Any other direct probability argument is to be regarded as of an *ad hoc* nature. Of course, statistical mechanics is itself founded on axioms which include probabilistic ones. Several very distinct sets of axioms for statistical mechanics have been examined in detail, ranging from the classical work of Fowler and Guggenheim¹⁰ to the modern and elegant treatment by Penrose¹¹. Such works have succeeded in deriving identical molecular partition functions from different sets of premises. These partition functions allow molecular distributions to be calculated by routine methods free from probability theory and from the need to question the axiomatic foundations in each individual instance. Such partition functions have not so far shown the axiomatic foundations to be at variance with their application to the physical world. On the contrary, they are supported by an impressive body of thermodynamic and spectroscopic measurements. Thus the second method of settling the controversy works upward from a level (partition functions) above which probability theory and *ad hoc* arguments are no longer required, whereas at a deeper, axiomatic level the status of partition functions has been secured by extensive work by mathematicians. The calculation of random ring-free f -functional polycondensation equilibria from partition functions for linear or non-linear polyatomic molecules (for example, ref. 10, equation 329, 2) is essentially of a routine nature. The translational factors of the partition functions are not relevant to condensed polymer systems and are omitted. The relevant factors are the rotational symmetry numbers. Provided there is free rotation of the bonds between repeat units, the symmetry number of the j th isomer in the x -mer fraction is proportional to the order $|G_{xj}|$ of the symmetry group (automorphism group¹²) of its molecular graph. Indeed the form of the equilibrium distribution of a polycondensate is not affected if all the species present are taken in their completely floppy ("graph-like") states¹³. The combinatorial entropy change of the reaction



is then derived from the partition functions, namely

$$\Delta S_c = R \ln |G_M|^x / |G_{xj}| \quad (5)$$

where M denotes monomer and P_{xj} the j th x -mer in the polymer distribution. The equilibrium constant of reaction (4) is

$$K_{xj} = w_{xj} / w_1^x N^{x-1} x \quad (6)$$

where N is the total concentration of repeat units, and w_{xj} the weight fraction of the j th x -mer. As proved from statistical mechanics by Gordon and Temple¹³, any phenomenological equilibrium constant can be factorized into two factors, one combinatorial and one reflecting fundamental driving forces. Absence of intramolecular reactions implies that every x -mer has $(x-1)$ inter-unit bonds, and equireactivity of functionalities shows that each of these bonds has the same fundamental entropy ΔS_b and enthalpy ΔH_b of formations

$$K_{xj} = (\exp \Delta S_c / R) K_b^{x-1} = (\exp \Delta S_c / R) (\exp (x-1)(T\Delta S_b - \Delta H_b) / RT) \quad (7)$$

Because this is valid for all x and j , the fundamental equilibrium constant K_b must be independent of what the functionalities are attached to, so K_b is related to the equilibrium concentration $Nf/(1-\alpha)$ of free functionalities and Nfa of reacted functionalities, thus

$$K_b = \alpha / (1-\alpha)^2 Nf \quad (8)$$

From the last four equations:

$$w_x = \sum_j w_{xj} = w_1^x x \{ \alpha / (1 - \alpha)^2 f \}^{x-1} |G_M|^x \sum_j |G_{xj}|^{-1} \quad (9)$$

The notion of a rooted tree is well known not only in graph theory (Harary¹⁴) but in its applications to chemistry since the work of Cayley in the nineteenth century. The notion occurs implicitly in previous methods of proving distribution (1) and in the attempt of Masson *et al.* to prove distribution (2). A molecule of the j th isomer of the x -mer fraction is turned into a graph by allowing the repeat units to form the nodes and the free functionalities to form the terminal points. Such a tree can then be rooted on any of its nodes and the branches ordered on the page to produce altogether T_{xj} , say, distinct node-rooted ordered forms, a process illustrated by Gordon and Temple¹³. From their theorem 2, which was established in a few lines, we obtain the special case

$$T_x \equiv \sum_j T_{xj} = x(f!)^x \sum_j |G_{xj}|^{-1} / f^{x-1} \quad (10)$$

The order of the automorphism group $|G_M|$ of the graph-like (floppy) monomer is $f!$, that is, the number of permutations of the f functionalities. Accordingly,

$$T_x = x |G_M|^x \sum_j |G_{xj}|^{-1} / f^{x-1} \quad (11)$$

and distribution (9) may be written

$$w_x = T_x w_1^x \alpha^{x-1} (1 - \alpha)^{-2x+2} \quad (12)$$

The number T_x of distinct node-rooted ordered x -mer trees is evaluated as a standard exercise in graph theory (compare Good¹⁵) without recourse to probability theory; T_x is the coefficient $C(\theta^x) \{ \theta N(\theta) \}$ of θ^x in the generating function

$$\theta N(\theta) = \theta(1 + u)^f \quad (13)$$

with

$$u = \theta(1 + u)^{f-1} \quad (14)$$

By Lagrange's expansion⁴

$$T_x = C(\theta^{x-1}) \{ N(u(\theta)) \} = (x-1)^{-1} C(\{ (u^{x-2}) \}) \{ N'(u)(1+u)^{f-1}(x-1) \} \\ = f(fx-x)! / (fx-2x+2)!(x-1)! \quad (15)$$

so that distribution (12) becomes

$$w_x = [f(fx-x)! / (fx-2x+2)!(x-1)!] w_1^x \alpha^{x-1} (1 - \alpha)^{-2x+2} \quad (16)$$

It follows from the normalization condition for w_x that $w_1 = (1 - \alpha)^f$ and this completes the proof of distribution (1) from standard statistical mechanics. Graph theory has merely served to evaluate the symmetry numbers occurring in partition functions.

Flory's account of gelation, now thirty years old, is sound, in spite of the attacks it suffers periodically. The state of matter near the gel point is of great importance not only to technology, but in biology^{16,17} as will be further illustrated in a forthcoming article (R. C. Hider and M. G., to be published).

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Reply to Gordon and Judd

ALTHOUGH expressions similar to that of Flory¹ have been derived by several authors for the size distribution in branched polymers it has been assumed, implicitly or explicitly, in such derivations that the conversion α equals the probability that a functional group chosen at random has reacted. We have shown that, because functional groups never occur singly after their mutual condensation, but always in pairs, the choice between a "reacted" and an unreacted functional group is not meaningful. Our choice must lie between unreacted groups and reacted pairs. Flory's incorrect use of α in this respect led immediately to a divergence at $\alpha = 1/(f-1)$ in his expression for the size distribution. This led Flory, and subsequently many other investigators, to the belief that this divergence corresponded to the gel point. It is hardly surprising that others with the same assumption derived the same result as Flory.

Gordon and Judd do not directly contest our explicit criticisms of Flory's method, but rather claim to have derived the Flory distribution by another method. Their method is based on the use of a "fundamental" equilibrium constant, independent of chemical combinatorics, to describe the driving force of a chemical reaction³. The combinatorial entropy change is calculated separately from the symmetry numbers in rotational partition functions, with the aid of graph theory. In equation (8) of their paper, however, Gordon and Judd express this fundamental equilibrium constant K_b in terms of the experimental quantity α . This procedure is incorrect because α , of necessity, already includes all combinatorial effects.

In our method of deriving the distributions it is assumed that each molecule possesses an unreacted functional group. Our procedure automatically excludes from consideration the case of $f=1$. That our treatment does not yield an acceptable result for this case is a measure of its consistency. We can, of course, readily modify our procedure to give the correct result for this case also.

The model common to all these treatments prohibits intramolecular condensation and therefore the point $\alpha = 2/f$ becomes a point of mathematical divergence. No characteristic of the model, however, requires a point of divergence at $\alpha = 1/(f-1)$, yet the normalization condition of Gordon and Judd can only be achieved in the range $0 < \alpha < 1/(f-1)$. A similar difficulty was encountered by Flory⁴ who attempted to rationalize the result by claiming that it applied to only a part of the polymeric system under consideration (that is, to the "sol fraction"). In this respect, Flory altered his model to accommodate the result. No such difficulty is encountered with our expression, which applies over the entire range of α which the model allows.

It is now clear that Flory's theory of gelation cannot be correct. Evaluation of size distributions in branched polymers

is important in silicate chemistry. Experimental evidence⁵ confirms that Flory's theory is inapplicable to such systems.

We thank Professor Gordon for sending us a copy of his manuscript before publication.

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BIOLOGICAL SCIENCES

Induction of Growth in Newt and Frog Limbs after Perfusion with Extracts from Newt Blastemas

It has been suggested that the nerves required for regeneration of the amphibian limb produce a "trophic material"^{1,2}. Lentz³ has suggested a "hormonal" role for the nervous system, but otherwise the nature of trophic material is undefined⁴.

From our experiments with lower organisms⁵⁻¹⁰, we found that the control of growth and differentiation is mediated through neurosecretion. For example, in *Hydra* small 25-35 Å particles, liberated by the nerve when large neurosecretory granules break down, participate in the control of growth and differentiation. Lentz³ has shown particles of identical size in nerves of regenerating newts after limb amputation. We propose that in regenerating amphibians, the neurosecretory granule breaks down and is then most concentrated in those dividing cells which continually provide new binding sites for it, namely the blastemal cells.

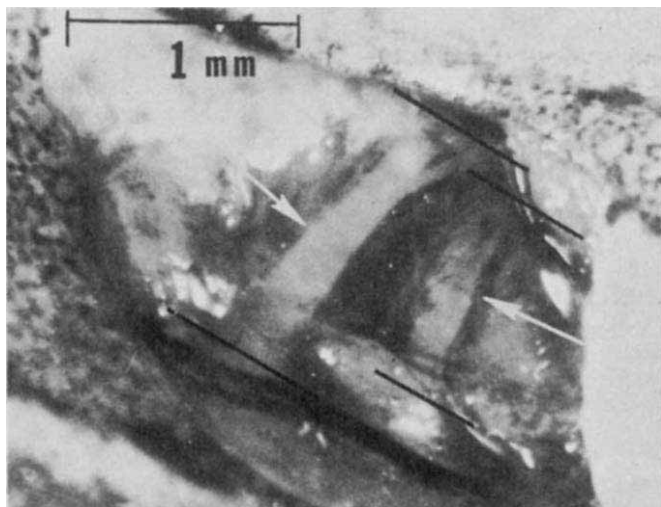


Fig. 1 A photograph of the brachial plexus (arrows) showing the two major nerves which constitute the plexus. A smaller third nerve of the plexus is not shown in the photograph. Lines show region of plexus removed by surgery ($\times 30$).

We have investigated this possibility using the newt *Triturus viridescens* (Lemburger, Wisconsin) and the frog *Rana pipiens* (Turttox Corporation, Chicago). Newts were kept at room temperature (25° C) in filtered aquaria and fed beef liver weekly. Frogs were kept in aquaria provided with a dry limestone shelf and fed twice weekly with *Tenebrio* larvae. The perfusion machine used was a Compact Infusion Pump, Model 975 (Harvard Apparatus Co., Inc., Massachusetts). Syringes (1 cm³ plastic disposable) were attached to 1/2 inch needles with 27 bore hole.

Newts from which blastemas were obtained were excised through the mid-humerus. Developmental stages of blastema formation were examined by making a pin prick and squeezing blastemal cells into a drop of Ringer solution and examining them under Nomarsky illumination or phase microscopy at $\times 1,000$. We found the number of mesenchymatous cells, which bind the 25-35 Å particle, to be greatest during a 20-30 day growth period following excision and we therefore used blastemas at this stage.

Blastemas were removed by cutting just distal to the initial excision site, and placed in a test tube surrounded by ice. Cold saline (4° C) was added to keep them moist during excision. The blastemas were micro-homogenized and then centrifuged in the cold (4° C) at 15,000 r.p.m. for 30 min. The supernatant was collected, diluted with 4.5 ml. of cold Ringer solution, and pipetted into aliquots of 0.3 ml. which were quick frozen in absolute alcohol/dry ice baths. An average of 392 blastemas was used for each preparation of extract. The frogs were excised mid-way along the humerus and treated as shown in Table 1.



Fig. 2 The emergence of a blastemal tip on an animal which was injected in the centre of the excised arm. Note the arrows which show the extent of growth ($\times 12.75$).

Perfusion of the frogs was begun 3 days after excision and continued every other day thereafter.

Denervation in the newts was accomplished not by simply excising the brachial plexus, but by complete removal of the nerve fibres (Fig. 1). Animals were denervated and sutured a day before the humerus was excised mid-way along its length. Naturally, it was important to reopen the animals periodically and determine if any nervous connexions had been established in the regions where the plexus had been removed. We found only one nerve connexion had been re-established and this particular animal was far behind in growth when compared with perfused animals.

Newts and frogs were tranquillized in a solution of tricaine methanesulphonate (MS222). Newts were perfused every other day with 0.05 ml. of extract over a 90 min period.

Six frogs were simply amputated along the humerus and

used as control animals. They were not perfused. In these animals the wounds healed within 1 week, with piled up connective tissue at the wound site; no growth ensued. Frogs injected with collagenase alone (2 mg/ml.) or with Ringer solution behaved in the same way. Frogs injected with blastemal extract showed minimal growth but did not form a blastema. Those injected with blastemal extract plus collagenase (2 mg/ml.) did not heal but began to show pronounced blastemas (Figs. 2 and 3). In Fig. 3 the blastema indicated by an arrow is about to emerge from the tip of the wound. In Fig. 4 (both photographed 30 days after amputation) a much more pronounced blastema is forming, but its area is limited. During the formation of this blastema, it was pricked with a pin and thousands of cells were squeezed out onto a drop of Ringer solution on a slide. Examination with Nomarsky and phase microscopy showed these cells to be identical to mesenchymatous cells found in the newt's blastema. These pictures were taken more than 1 month before this writing and the blastemas are still growing.

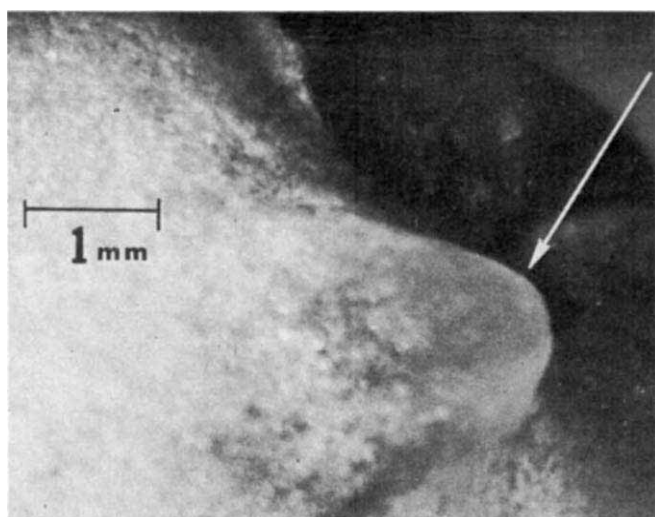


Fig. 3 A pronounced blastema in the frog which had been perfused basally with extract. Note the pronounced growth at the injection site. This tip of growth was found to contain a solid mass of mesenchymatous cells. The growth has continued for the past month and all indications are that a small limb will be regenerated ($\times 17.25$).

If the needle is inserted directly through the centre of the wound, blastemal growth is general but smaller in length (Fig. 2). If the needle is inserted towards the edge of the wound, blastemal growth is regionalized and pronounced (Fig. 3).

Table 1 Treatment of Frogs

Animal	Type of perfusant	Amount (ml.)	Period of perfusion (min)
Group 1			
1	Ringer solution	0.1	90
2	Collagenase (0.2 mg) dissolved in Ringer solution	0.1	90
3	Collagenase (0.2 mg) dissolved in extract	0.1	90
4	Extract	0.1	90
Group 2			
5	Ringer solution	0.1	90
6	Collagenase (0.2 mg) dissolved in Ringer solution	0.1	90
7	Collagenase (0.2 mg) dissolved in extract	0.1	90
8	Extract	0.1	90

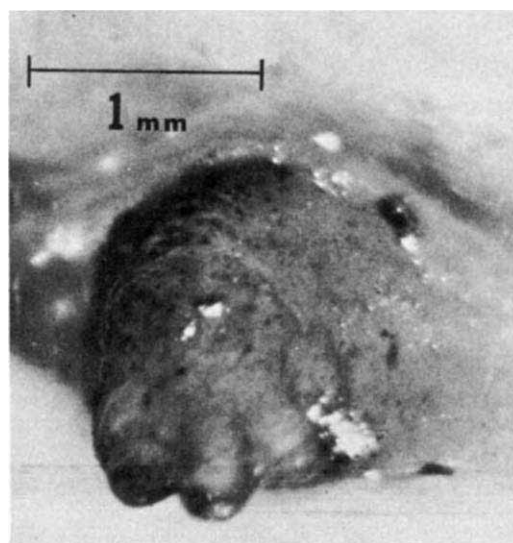


Fig. 4 The regrowth of a newt limb where nerve connexions were excised at the level of the brachial plexus. Since this photograph was taken, the animal has continued to form fully differentiated digits. In this photograph only the finger stubs are shown ($\times 30$).

In newts, for three animals used in each trial, perfusion with Ringer solution not only inhibits growth but can cause the arm to deteriorate back to the shoulder girdle. Animals perfused every other day with a concentrated extract will be stimulated to maintain normal regeneration. In Fig. 4 the animal was photographed just as the digits were emerging at 2 months. It is important to note, however, that a well defined blastema was formed after the first 5 days of perfusion on all the animals receiving the extract. Control animals whose nerves have been excised and not perfused are exactly as those described by Deck and Futch⁴. In the beginning it seems that some blastemal growth is taking place, but this reaction soon ceases. The thickened epidermis and bulbous tip at the excised tip remain unchanged for 50 days.

We believe that the active material or inducer is a product of breakdown of a neurosecretory granule (demonstrated in newts by Lentz, but present in the same size and form in nerves of virtually every phylum examined with the electron microscope). We postulate that this compound is probably a small polypeptide in the same family as that found in coelenterates and flatworms. Our experiments will be repeated with perfusate separated by column separation followed by HVE paper separation; we hope that the substance which will promote normal growth in the absence of nerve in amphibia will also be applicable to higher animals.

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Cell-free Transmission of Mouse Neuroblastoma

AFTER the description of the cell-free transmission of neuroblastoma C1300, a transplantable tumour in strain A/J mice, by Prasad *et al.*¹, we made ten attempts to reproduce their results, first using their extraction procedure and then by using the viral concentration procedure of Moloney², modified by Huebner *et al.*³. We inoculated twenty-eight prenatal, forty-seven newborn, and sixteen 4-week-old A/J mice but failed to obtain positive results, even after 100 days postinoculation. Because we used newborn mice that are usually more susceptible to oncogenic viruses than the older mice used by Prasad *et al.*, and viral concentrates rather than filtrates, we expected to increase our chances of transmission.

We have found numerous intracisternal A-type particles and rare budding C-type particles in C1300 tumours, confirming the results of Prasad *et al.* In addition, we have found specific antigens of C-type RNA tumour viruses by the complement fixation test. A/J mice, like many other inbred and outbred strains of mice, are known to carry wild-type infectious C-type RNA viruses in spontaneous and transplanted non-leukaemic tumours, but except for the FBJ virus, no C-type RNA virus from solid tumours of any mouse strain has been shown to induce solid tumours of the original type.

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Effects of Amphetamine on Single Cell Activity in a Catecholamine Nucleus, the Locus Coeruleus

THE action of amphetamine on the central nervous system has been related to its ability to release and inhibit the re-uptake of dopamine (DA) and noradrenaline (NA) at presynaptic terminals¹⁻⁴. Abundant catecholamine (CA) stores have been demonstrated in the cells of the locus coeruleus⁵⁻⁷. These two observations suggest that amphetamine causes the release and decreased re-uptake of CA from locus coeruleus cells. The drug, by promoting the accumulation of CA postsynaptically, might thereby initiate neuronal feedback inhibition of locus coeruleus units; such a mechanism has been postulated to explain biochemical observations on CA systems treated with amphetamine⁸. The opposite effect, feedback stimulation, has also been postulated to explain observations of the CA blocking effects of neuroleptics (chlorpromazine and haloperidol)⁹⁻¹¹.

To evaluate these hypotheses, extracellular microelectrode recordings were made from single neurones in the rat locus

coeruleus, before and after treatment with amphetamine. Chlorpromazine was also given to evaluate its anti-amphetamine effects; such effects have been described previously in both clinical¹² and behavioural settings¹³⁻¹⁵.

We used male Charles River rats weighing 225–300 g. Recordings were made by techniques similar to those previously described¹⁶. Animals were anaesthetized with chloral hydrate (400 mg/kg, intraperitoneally) and mounted in a stereotaxic apparatus. Coordinates, representing the outer dimensions of the locus coeruleus, were estimated by direct measurement from serial pontine sections of the brain from a Charles River rat, sectioned along the axes of König and Klippel¹⁷. A 3 mm burr hole was made at coordinates within the range of lateral 1,000–1,300 μ m and posterior 1,700–2,200 μ m. Tungsten microelectrodes with tip diameters of 1 μ m were lowered through the burr hole by a hydraulic microdrive. Signals were passed through a high input-impedance amplifier and displayed on an oscilloscope. Unit rates were measured by an electronic counter whose analogue output was traced graphically by a potentiometric recorder. Signals from the oscilloscope (700–2,000 Hz) also drove an audio monitor.

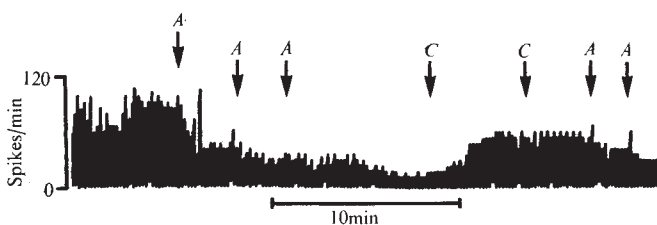


Fig. 1 Typical response of a locus coeruleus unit to amphetamine (A) and chlorpromazine (C). Spontaneous rate was 90 spikes/min. The record consists of the number of spikes counted over consecutive 10 s intervals. Within 30 s after the intravenous injection of amphetamine (0.2 mg/kg), a marked decrease in rate was observed. Two subsequent doses of amphetamine (0.4, 1.0) produced additional slowing (to 12 spikes/min). A recovery of rate to two thirds of the original was observed within 1 min after i.v. injection of chlorpromazine (1.0 mg/kg). Additional chlorpromazine (1.0) had no additional effect. Subsequent doses of amphetamine (0.5, 1.0) were less effective in decreasing the rate than in the untreated animal.

Only cells with a stable rate and a uniform spike amplitude were used. The waveform was observed for 5–10 min. Drugs were then administered intravenously to the tail in 0.05–0.2 ml. volumes given slowly over a period of 5–10 s. *d*-Amphetamine sulphate and chlorpromazine hydrochloride were given dissolved in distilled water at concentrations of 0.25 mg/ml. or 2.5 mg/ml. Only one unit was studied in each rat to avoid possible residual drug effects. At the completion of an experiment, the location of the electrode was marked by making an anodal electrolytic lesion (5 μ A for 60 s). Animals were perfused through the left ventricle of the heart with a solution of 5% glutaraldehyde in saline. Serial frozen sections were cut at 50 μ m and stained with cresyl violet. The location of each recording electrode was precisely identified in this manner. Fluorescent histochemical studies of the brain were carried out using the techniques described before^{5,18-20}.

Recordings were made from single units in thirty-five rats; subsequent histological examination showed that ten of these were in the locus coeruleus, and in these spontaneous activity decreased to less than one third of the original rate after small intravenous doses of *d*-amphetamine (Fig. 1). A marked response to amphetamine occurred with doses as small as 0.2 mg/kg. Larger doses produced further depression, the maximum depression of firing being achieved with 0.5–1.5 mg/kg (Fig. 1). Although in each recording a single prominent unit spike was monitored, multiple "background" spikes from neighbouring locus coeruleus cells also showed a gross reduction in frequency with amphetamine.

The twenty-five units recorded from regions outside the locus coeruleus were situated in the ventral lateral midbrain, central grey, pontine and mesencephalic reticular formations, and the ventral folia of the cerebellum. In fourteen (56%) of these, the rate more than doubled; in another four (16%) the rate decreased to one half to one third of the original with amphetamine doses from 0.25–3.7 mg/kg; and in seven (28%) there was no significant change in rate. In no case did a non-locus coeruleus cell show a reduction in firing rate to less than one third the spontaneous rate. Thus, the dramatic slowing response seen in locus coeruleus units after amphetamine treatment was unique among the units studied.

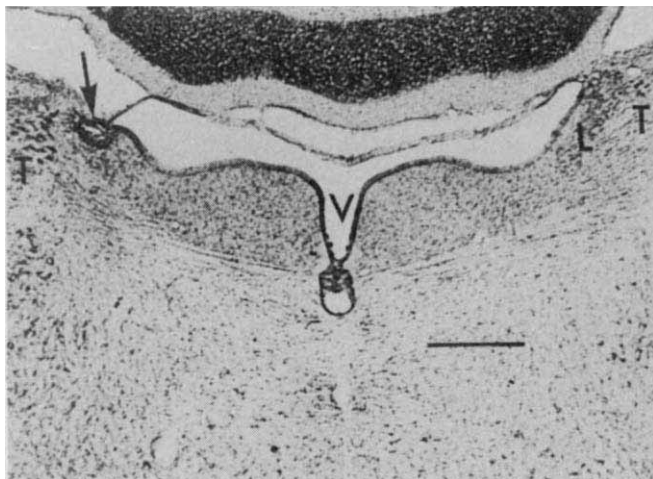


Fig. 2 Transverse histological section showing an electrolytic lesion (5 μ A, 60 s) made through a recording electrode in the locus coeruleus on the left (arrow); a normal locus is seen on the right (L). V, Fourth ventricle; T, mesencephalic nucleus of the trigeminal nerve. Cresyl violet staining; scale, 0.5 mm.

Chlorpromazine (0.2–1.5 mg/kg) was given after amphetamine pretreatment in seven of the ten recordings from locus coeruleus units. In four of these there was a prompt return to nearly normal activity 1–2 min after intravenous chlorpromazine treatment (0.2–1.0 mg/kg). In these four units the effects of chlorpromazine were found to be partially reversible by additional amphetamine treatment, but the response to amphetamine was attenuated after chlorpromazine had been given and the depression of firing rate was only transient (Fig. 1). In the remaining three units pretreated with amphetamine there were only small or transient increases in activity after chlorpromazine treatment (0.8–1.5 mg/kg).

Neurons of the locus coeruleus differed from surrounding cells in their responses both to pharmacological stimulation and to somatic stimulation. In rats anaesthetized with chloral hydrate, locus units were found to have a slow rate of 1 to 2.5 spikes/s with a regular rhythm. In the immediate vicinity of the locus (within 500 μ m), three additional cell types were often observed, one of which responded to touch. Some of these cells were sensitive to deep pressure and showed a two to three-fold increase in spontaneous rate post-stimulus; others were sensitive to light touch and showed transient bursts of discharges immediately after a stimulus. The second cell type was found to discharge in synchrony with respiration. In several of these cells there were bursts of 8–10 spikes before each inspiratory movement. Cells of the third type had firing rates which increased and decreased in very regular cyclic patterns (1–0.3 cycles per min). These cycles were not influenced by tactile, aural or visual stimuli but were markedly disrupted by small doses of amphetamine (0.05–0.5 mg/kg). These three cell types were different to locus coeruleus units, which were not synchronized with respiration and which rarely

responded to tactile stimuli (two units showed transient post-stimulus suppression after deep pressure applied to the hind toes).

Cresyl violet-stained sections were used to identify the location of every cell from which a recording was made. This identification depended on microscopic examination of anodal lesions through the recording electrodes (Fig. 2). Our fluorescent histochemical results are consistent with the descriptions by Dahlström and Fuxe of their group A6 cells⁵. The histological appearance of group A6 is identical to the appearance of the locus coeruleus in routine cresyl violet stained sections (Figs. 2 and 3).

Our results show that amphetamine in small doses (0.2–0.5 mg/kg) produces a large decrease in the spontaneous rate of firing in units of a catecholamine nucleus, the locus coeruleus. A dose of 0.2 mg/kg decreases spontaneous activity to one half to one quarter of the original rate. Additional amphetamine usually produces additional depression of activity with a maximum effect being achieved at levels of 0.5–1.5 mg/kg. Although a small percentage of cells not in the locus coeruleus showed a decrease in spontaneous activity with doses between 0.5 and 2.0 mg/kg, the decrease never exceeded two thirds of the original activity. In contrast, locus units could always be depressed beyond this level with similar doses. Most cells outside the locus showed either an increase in rate or no change at all after amphetamine treatment (0.05–3.5 mg/kg). Excitatory effects of amphetamine administered parenterally in other regions of the brain^{21,22}, including some serotonin-containing cells of the midbrain raphe nuclei²³, have been described already.

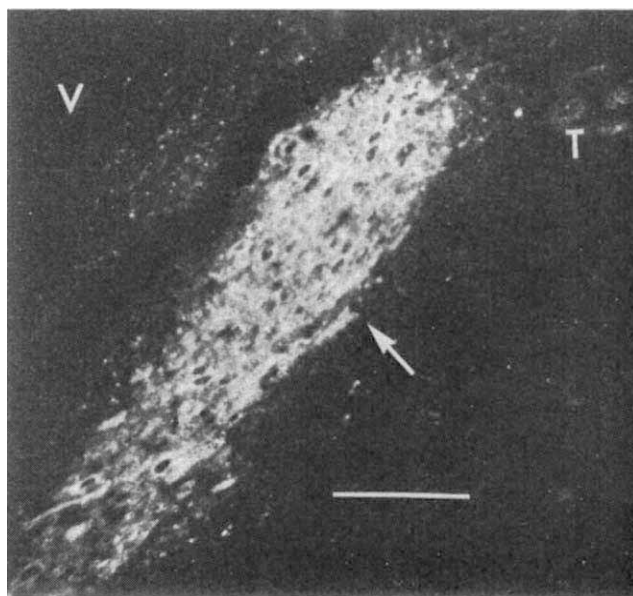


Fig. 3 Fluorescence micrograph of locus coeruleus (arrow) in normal rat brain. The locus parallels the floor of the fourth ventricle (V). Three faintly autofluorescent cells of the mesencephalic nucleus of the trigeminal nerve (T) are seen adjoining the nucleus along its dorsal lateral border; scale, 0.1 mm.

The responses to chlorpromazine were less uniform than those to amphetamine. In four out of seven locus coeruleus cells in which chlorpromazine (0.2–0.4 mg/kg) was given, a prompt and nearly complete reversal of amphetamine effect was observed. Additional increases in rate were not observed with total doses exceeding 1.9 mg/kg. These responses to chlorpromazine were transiently and only partially reversible with further doses of amphetamine. The remaining three cells showed only small or transient increases in rates after chlor-

promazine (0.5–2.0 mg/kg) treatment. It is not yet clear why there was a variable response to chlorpromazine. Nor is it known what effect chlorpromazine has on spontaneous activity of locus cells in the absence of amphetamine.

The mechanism by which amphetamine reduces the rate of firing in cells of the locus coeruleus might be related to a direct action on the locus cells or to an action at some other central or peripheral site. On the basis of biochemical observations, it has been proposed that, in the central nervous system, amphetamine releases presynaptically bound NE or DA and blocks their re-uptake^{1–4,24,25}. Amphetamine treatment would be expected to promote the pre-synaptic release and post-synaptic accumulation of catecholamines at locus coeruleus terminals. Our results are consistent with the suggestion that by promoting the pre-synaptic release of catecholamines with amphetamine, a feedback inhibition of CA neurones can be produced⁸.

Several other hypotheses concerning the action of amphetamine should also be considered. It might function as a sympathomimetic amine^{26,27}, acting directly in the central nervous system. If it does, the results from this experiment would suggest that reduction of the rate in locus cells is caused by either a direct inhibitory response to sympathetic input or an indirect feedback inhibition from a post-synaptic neuronal circuit. Pretreatment with α -methyl tyrosine, an inhibitor of CA synthesis, may be of use in distinguishing between these direct and indirect sympathomimetic actions. Another mode of amphetamine action might be related to an induced alteration of neuronal input to the locus coeruleus. One type of altered input might be secondary to peripheral adrenergic effects of amphetamine, but this hypothesis seems unlikely in that peripheral adrenergic effects are negligible after small doses of amphetamine (0.2–1.0 mg/kg)²³. Finally, some unique action of amphetamine on locus coeruleus cells is a possibility which has not been ruled out.

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Influence of Histo incompatibility between Mother and Foetus on Placental Size in Mice

ALTHOUGH the mammalian foetus is a natural allograft because it inherits paternal strain antigens histoincompatible with the mother, frank manifestations of rejection do not occur. (Explanations for this phenomenon have already been reviewed^{1–7}.)

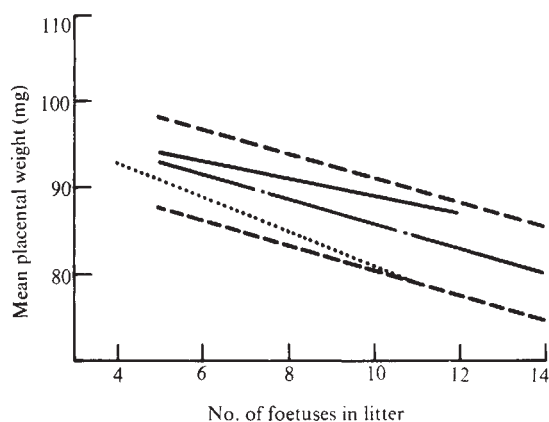


Fig. 1 Least squares regression lines of mean placental weights on numbers of foetuses in isogenic (—), congenic (---) and allogeneic (— · —) crosses (groups I, II and III respectively). Equations of least squares regression lines, and standard error of estimate, for each group are as follows: Group I: $y = 100.47 - 1.45x \pm 5.30$; group II: $y = 101.01 - 1.99x \pm 4.72$; group III: $y = 98.95 - 1.02x \pm 3.91$. Standard error for the isogenic group is indicated graphically (- - -).

Placental size in mice tends to increase when mother and foetus are histoincompatible⁸. When two inbred strains, C57BL (H-2^b) and A2G (H-2^a), were crossed, placentae of F₁ foetuses were significantly larger than those from corresponding isogenic crosses. Transplantation of homozygous blastocysts from one strain into the uteri of pseudopregnant females of the allogeneic strain showed that this difference is attributable to antigenic dissimilarity rather than heterozygosity⁸. This effect was also observed in crosses of C57BL females and C3H (H-2^k) males⁹. Furthermore, C57BL mothers immunized against paternal A2G antigens produced hybrid progeny with larger placentae than those of corresponding hybrid progeny from non-immunized mothers^{10,11}. Moreover, mothers rendered tolerant at birth to paternal strain antigens produced placentae smaller than those from untreated mothers bred to allogeneic males, and virtually equivalent in size to those from control inbred litters. Crosses of *Peromyscus maniculatus*, the deer mouse, and *P. polionotus*, the oldfield

mouse, however, showed that placental weights of fetuses from reciprocal hybrid crosses differed significantly from each other as well as from the parental types, and that the mean placental weight for one of the hybrid combinations was significantly smaller than that of the corresponding inbred mating¹².

In an attempt to correlate quantitatively the amount of placental enlargement with the degree of histoincompatibility between parental strains of mice, young adult virgin females of the C57BL/10Sn (H-2^b) strain were divided into three groups and mated at approximately 90 days of age to isogenic males, males of the congenic B10.A (H-2^a) strain, and males of the allogeneic A/J (H-2^k) strain, groups I, II, and III, respectively. Females were killed 17.5 days after the appearance of the vaginal plug. The uterus was removed and placed on filter paper moistened with normal saline. Foetoplacental units were removed from the uterus and then foetus, foetal membranes and umbilical cord were cut away from each placenta. Placentae were then gently blotted between two saline-moistened filter paper disks and weighed to the nearest milligram on a spiral spring balance. No significant difference was found between mean placental weights of litters with zero and one

Studies in the rat have shown that excess heterozygosity for Ag-B histocompatibility antigens among backcross progeny is due to increased homozygote susceptibility to postnatal wasting disease resembling a classic graft-versus-host reaction^{13,14}. It was suggested that histoincompatibility at minor transplantation loci is responsible for this decreased postnatal survival and that superimposition of histoincompatibility at the major Ag-B locus reduces this selective disadvantage, perhaps by a mechanism involving immunological enhancement and steric hindrance¹⁴.

Thus, in spite of the lack of statistical significance of the data in Table 1, there are indications that heterozygosity and consequent histoincompatibility with the mother at the major H-2 transplantation locus may confer on the foetus a selective prenatal survival advantage. If the mechanism proposed here operates in a similar fashion during gestation in mice, then the similarity of the percentage survival values for the progenies of the isogenic and allogeneic crosses may be due to compensation in the latter by superimposition of H-2 incompatibility on non-H-2 incompatibilities and hence enhancement of survival to the isogenic histocompatible level.

Table 1 Foetal Survival and Histocompatibility

Group	Mother	Foetus	No. of litters	No. of implantations	Mean No. of implantations per litter and s.d.	Survival (%) of implanted conceptuses to day 17.5 of gestation
I	C57BL	C57BL × C57BL	30	241	8.03 ± 2.98	87.6 (211/241)
II	C57BL	C57BL × B10.A	31	259	8.35 ± 2.20	86.1 (223/259)
III	C57BL	C57BL × A/J	29	251	8.66 ± 2.14	91.2 (229/251)

reabsorbing foetus, so both litter categories were included in the statistical analysis. Litters containing two or more reabsorbing fetuses were not included in the statistical analysis. Litters are considered in terms of the number of live fetuses rather than the total number of implantations, after McLaren⁹. Breeding was continued until data for twenty litters of each of the three crosses were accumulated. The mean placental weight for each litter was plotted according to the number of fetuses in the litter. Regression lines were fitted to these data by the method of least squares. The relation of mean placental weight to the number of fetuses in the litter for the three crosses is shown in Fig. 1.

Litters from the three crosses show a negative regression of mean placental weight on increasing litter size. There is no significant difference in the positions of the three regression lines nor are their slopes significantly different.

Thus, in this study, the H-2^b × H-2^a cross produced no significant increase in placental size relative to the corresponding isogenic cross, whereas in previous experiments such increases were observed^{8,10,11}. Although the paternal strains, A/J and A2G respectively, are immunogenetically identical at the major H-2 histocompatibility locus, they are certainly not identical at all minor histocompatibility loci. Indeed, if the phenomenon of placental enlargement were attributable to histoincompatibility at these minor loci, then the difference in results between this study and previous ones might be explained by such a mechanism, rather than by invoking differences in experimental techniques or statistical methods.

There was a tendency to an increased number of implantations per litter with increasing histoincompatibility between mother and foetus. There was also an increased percentage survival of implanted conceptuses to day 17.5 of gestation for progeny of the allogeneic cross compared with those of the isogenic cross. But neither of the tendencies is statistically significant (Table 1).

The design of this study did not permit investigation of possible deleterious effects of minor locus histoincompatibility in the absence of superimposed H-2 incompatibility. Experiments are now in progress to evaluate this proposed effect and the combined effects of major and minor locus histoincompatibilities on both prenatal and postnatal viability of heterozygous progeny.

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Relationship between Antibody-mediated Immunosuppression and Tolerance Induction

ANTIBODY-MEDIATED immunosuppression is no longer thought to be solely due to the masking of antigen by antibody. The hypothesis that events beyond the binding of antibody to antigen are operative in antibody-mediated immunosuppression is supported by the following facts. (1) Antigen-masking need not be complete to obtain pronounced suppression of immune responses^{1,2}; (2) interactions between antigenic determinants on the same immunogenic particle (molecules to whole cells) take place so that suppression of immune responses to one antigenic determinant may occur with the binding of antibody to another non-cross-reacting and topographically separate antigenic determinant³⁻⁵; (3) parts of the antibody molecule, other than that involved in the binding of antigen, are important in the regulation of the immune response by antibody⁶⁻⁹; (4) immunosuppression does not necessarily become more pronounced when the dose of passively administered antibody is increased but proceeds through an optimum beyond which less suppression is observed^{10,11,13,14}; and (5) immunosuppression by antibody can lead to inactivation of the antigen-sensitive cell population¹²⁻¹⁴. Similarly, immunological tolerance is no longer thought to be the simple elimination of immunologically competent cells by antigen. The following data suggest a more complex mechanism in the induction of immunological tolerance. (1) Animals which demonstrate tolerance seem to possess both sensitized lymphoid cells and soluble factors (antibodies or antigens) which prevent the sensitized cells from functioning^{15,16}; (2) induction of tolerance is often preceded by the production of measurable antibody³¹ or antibody-forming cells^{17,18}; (3) induction of tolerance requires the presence of a functioning complement system¹⁹; and (4) cells capable of binding antigen remain, or may increase, during the attainment of the tolerant state²⁰⁻²³. Consequently, it now seems possible that these two hyporeactive states, antibody mediated immunosuppression and immunological tolerance, share common characteristics and common modes of induction.

Teams in two laboratories have demonstrated the optimal effect with regard to immunosuppression by antibody^{10,11,13,14}. In both cases, optimal immunosuppression was achieved by certain doses of antibody; if more antibody was added, the degree of immunosuppression became less. In Diener and Feldmann's experiments^{13,14}, the optimal effect was demonstrated in terms of the degree of inactivation of immunocompetent cells, whereas we assessed the degree of suppression of the 19S antibody-forming cell response^{10,11} and of 7S immunological "memory" on cell transfer¹⁰. In both experimental systems^{10,11,13,14}, the quantity of antibody which gave the optimal suppression was likely to be less than that needed for complete covering of all antigenic determinants. In these experiments, the suppressive antibody was administered passively, but data are available to suggest that endogenously produced antibody is capable of inducing feedback regulation of immune responses²⁴.

Fig. 1 indicates the relationships which have been reported to exist between dose of antigen and either (1) the concentration of antigen in tissues or (2) the amount of antibody which is formed in the initial immune response. The concentration of antigen in tissue bears a simple linear relationship to the amount of antigen which is administered^{25,26}. On the other hand, the amount of antibody formed initially does not follow a simple straight line antigen dose-response relationship, but a sigmoidal or plateau response characteristic of many biological responses²⁸⁻³¹. Because of the straight line relationship for antigen in tissues and the sigmoidal relationship for initial immune response, four phases or zones should be discernible as one proceeds from low to high doses of antigen. There is a threshold dose of antigen below which no initial immune response is induced. This is followed by a zone where the produc-

tion of antibody is minimal and insufficient to react with all the tissue antigen. As the dose of antigen is increased further there is a rapid increase in the amount of antibody formed initially so that antibody excess is attained. With even greater doses of antigen, the amount of antigen in tissues will be in excess even if the initial antibody response remains maximal. Therefore, there is a low and high zone of antigen doses where antigen in tissues would be in excess of the antibody produced initially, while there is an intermediate zone where the concentration of antibody would be in excess of the amount of antigen in tissues. From experiments indicating that certain low levels of passively administered antibody which do not saturate antigen suppress immune responses maximally^{10,11,13,14}, one could deduce that the zones of tissue antigen excess over antibody produced would lead to suppression of immune responses (tolerance), whereas the zone of antibody excess would allow continuation of immune responses. Such a relationship between immunological responsiveness and dose of antigen has been reported by Mitchison³¹.

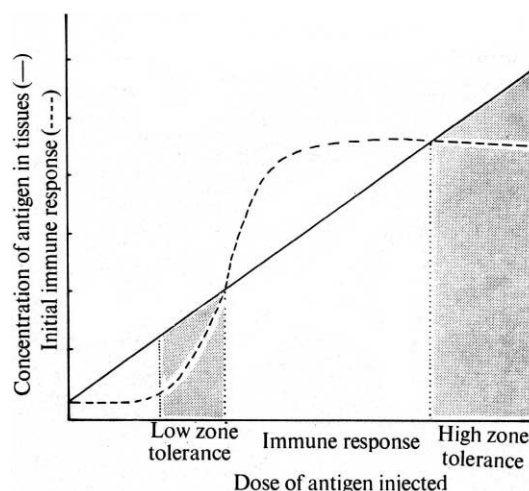


Fig. 1 Relationship between dose of antigen and either concentration of antigen in tissues (—) or level of initial antibody response (---). Zones where antibody is present but not in excess of antigen in tissues represent tolerizing conditions ("low and high zone tolerance"), whereas the zone where antibody is present and in excess of antigen in tissues allows a continuance of the immunologically responsive state ("immune response"). A dose of antigen which leads to the presence of tissue antigen but no induction of the immune response does not alter the immune capacity.

In summary, we propose that immunological tolerance develops when an immune response is regulated by the presence of antigen-antibody complexes in relative antigen excess. This theory accounts for the following observations: (1) low and high zone tolerance with an intermediate zone of heightened immunological responsiveness, (2) antibody or general immunoglobulin formation in the early phases of tolerance induction, (3) the role of complement indicating the presence of complement-fixing antigen-antibody complexes in the induction of tolerance, (4) antibody and immunologically competent cells present at some time in classically tolerant animals (the antibody and cells may disappear in long standing tolerance), and (5) evidence which suggests that normally reactive cells become inactivated when injected into tolerant animals.

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Thymus-Marrow Cell Interaction evaluated by PHA Stimulation and Graft versus Host Activity

THERE is now abundant evidence for cooperation in certain immune responses between B and T lymphocytes¹ which are respectively of bone marrow and thymus derivation. B cells have been shown to secrete antibody but the helper role of the T cell is less well defined. It has also been suggested that in mice PHA responsiveness can be an exclusive characteristic of T cells². Experiments will be presented in this paper which can be interpreted to indicate that the capacity of dissociated thymus cells to respond to phytohaemagglutinin (PHA) *in vitro* can be much augmented by the presence of bone marrow cells which could be thought to have a helper role in this respect. A small series of experiments is also presented which suggests that a similar form of cooperation can take place in graft versus host responses.

The basic design of experiment involved culture of newborn mouse thymocytes and adult mouse bone marrow in the presence of PHA. In the parallel series of experiments the same kinds of parental cells were injected singly or in combination into F₁ hybrid recipients in order to induce a graft versus host reaction.

CBA, C57BL and (CBA × C57BL)F₁ hybrid mice of both sexes were used as thymus and bone marrow donors. Bone marrow cells obtained from femurs and tibiae of normal 4-6 month old animals were diluted to 2×10^6 viable cells/ml.

in Hanks-Eagle minimal essential medium (MEM), containing 20% foetal calf serum (FCS, Gibco). Intact thymus lobes from newborn (2-7 days) and adult (4-10 months) animals, cleaned to avoid mediastinal lymph node contamination, were gently pressed through a multilayer nylon sieve and cell concentration was fixed at 2×10^6 viable cells/ml. in MEM. Triplicate cultures with and without 0.025 ml. PHA (Wellcome), containing $2-6 \times 10^6$ cells in a standard volume of 3 ml. MEM, were prepared from thymus and bone marrow cell suspensions (group A). Mixtures of both in varying ratios were also set up (4:1 to 1:4) (group B). Another group of cell mixtures (group C) was prepared according to a modified technique for one-way directional cultures by using mitomycin C (30 µg/ml., A. Christiaens s.a., Brussels) treatment to inhibit DNA synthesis of one of the two cell populations in culture³. Cultures were kept for 24-96 h in a 5% CO₂ atmosphere at 37° C. DNA synthesis was determined in all the groups by adding 1.5 µCi/ml. ³H-thymidine (³H-TdR) 6 h before harvesting and measuring the radioactivity in a liquid scintillation spectrometer^{3,4}.

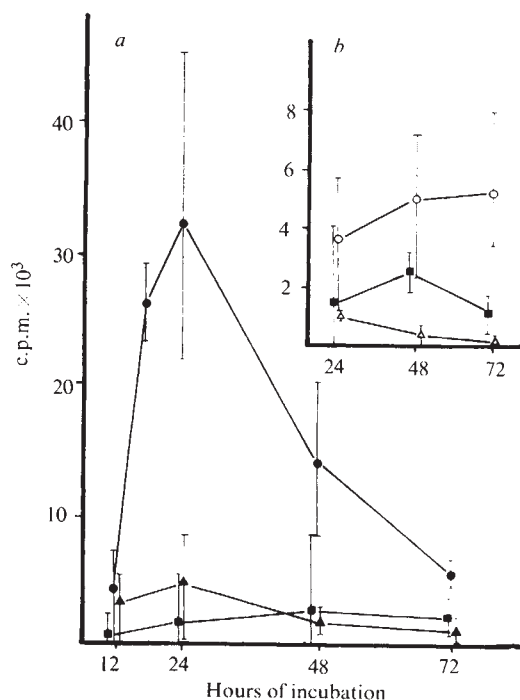


Fig. 1 ³H-TdR incorporation induced by PHA in thymus-marrow cell mixtures. Values refer to 4×10^6 thymocytes and 2×10^6 unfractionated marrow cells/culture (c.p.m. × 10³): difference in incorporation between PHA-stimulated cultures and the respective unstimulated controls (PHA-C). ■, Bone marrow; ▲, newborn thymus; △, adult thymus; ●, newborn thymus + bone marrow; ○, adult thymus + bone marrow.

In parallel with these *in vitro* assays, differing amounts of CBA thymocytes, bone marrow or both, mixed in different proportions, were injected into F₁ hybrid litters in order to evaluate possible increments in graft versus host activity of the mixtures (group D). Spleen index was calculated on day 9 and values above 1.30 were considered positive.

When newborn thymus cells were mixed in various proportions with syngeneic bone marrow, a remarkable increase of thymidine uptake in PHA stimulated cultures was observed. This response depended on the number of thymocytes in the mixture and the optimal thymus marrow concentration was $4 + 2 \times 10^6$ cells/culture; peak response was usually detected at 24 h (Fig. 1a). But when adult thymocytes were mixed similarly with marrow cells, a slight increase in ³H-TdR uptake was observed, which was not statistically significant (Fig. 1b).

Data obtained from one-way directional cultures show that the PHA response of unblocked cell mixtures and mitomycin-blocked marrow cells in culture with intact thymocytes is strikingly similar. Highest response was achieved in both mixtures when a 2:1 thymocyte:marrow ratio was employed within the limit of 4×10^6 thymus cells/culture (Fig. 2). Beyond this limit a significant response was still registered, albeit reduced. No significant increase in PHA response was seen when blocked thymocytes were cultured with intact marrow cells. Moreover, an increase in the number of blocked marrow cells over 2×10^6 cells/culture did not further enhance the response of a fixed number of unblocked thymocytes.

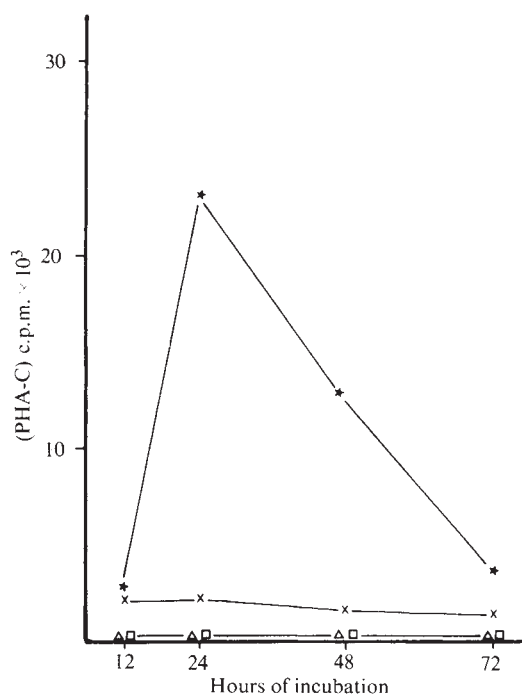


Fig. 2 ^3H -TdR incorporation induced by PHA in thymus-marrow, one-way directional cultures. Δ , (NT) (newborn thymus); $\square$, (BM) (bone marrow); $\star$, NT+(BM); $\times$, (NT)+(BM); $\circ$, mitomycin-treated.

Finally, Table 1 summarizes some of the results obtained by the Simonsen assay, by injecting thymus and marrow cell suspensions mixed in various proportions or administered as single cell suspensions. It is evident that bone marrow cells are devoid of graft versus host activity at the doses employed, while immunocompetent cells of this type are detectable in newborn thymus cell suspensions. In most cases, however, the respective thymus-marrow cell mixtures showed an increase well above the expected sum of the spleen indices obtained by injecting the same cell suspensions separately. No graft versus host activity was expressed by adult thymocytes injected separately or in mixture with marrow cells.

No fully satisfactory explanation can be offered for our findings. It seems that cells from the thymus of newborn mice can respond to PHA but only to any significant degree in the presence of syngeneic bone marrow cells. Previous experiments^{5,6} have indicated that a glass adherent cell population derived from bone marrow is less effective in enhancing the response of newborn thymocytes which suggests that the effect observed is not macrophage-mediated. The response observed by newborn thymocytes in contact with adult bone marrow plus PHA was different in that the peak of tritiated thymidine incorporation occurred at 24 h rather than at 48 h which is characteristic of mouse peripheral blood lymphocyte populations in our system. It could be argued that a newborn

mouse thymus contains more "mature" cells about to emerge into the periphery where they would form part of the PHA-responsive lymphocyte pools than does an adult mouse thymus. The helper role of the bone marrow has to be elucidated further and is difficult to relate to T and B cell cooperation theories. It is still uncertain whether bone marrow and thymus contain the mature form of either cell species. Thus, in a further series of experiments we shall seek similar synergism between T and B cells derived from secondary rather than primary lymphoid organs.

Table 1 Immunocompetence of Thymus and Bone Marrow Cell Mixtures

Cell type	Cell dose ($\times 10^6$)	Mean	Range	Spleen index *	
				Positive tests/total	%
Bone marrow	10	1.06	(1.01-1.15)	0/8	0
	20	1.06	(0.77-1.17)	0/8	0
	30	1.03	(0.99-1.12)	0/4	0
Newborn thymus	10	1.14	(0.97-1.49)	1/4	25
	15	1.30	(1.02-1.83)	3/9	33
	20	1.52	(1.06-2.00)	13/18	72
Bone marrow and newborn thymus	10+10	1.61	(1.23-2.25)	8/10	80
	10+15	1.55	(1.28-1.87)	13/15	87
	10+20	1.81	(1.44-2.12)	8/8	100

* Calculated according to Simonsen⁶ at day 9. Results refer to 108 animals injected with cells and fifty-four controls injected with medium. Values over 1.30 were considered positive.

The close similarity between the results of PHA stimulation and graft versus host assay by thymus-marrow combinations is intriguing but faces many of the interpretative problems already alluded to. It hints, however, at the possibility that certain kinds of PHA responsiveness can be used as a measure of the capacity to engender the sort of cell-mediated immune responses which are involved in graft versus host reactions.

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Multiplication of the Scrapie Agent

ALTHOUGH the transmission of scrapie by the inoculation of filtrates of tissue from affected animals suggests that the agent multiplies in the cells of the host, there is little published evidence of its propagation in cell cultures. Gustafson and

Kanitz¹ reported that cell cultures could be established more readily from scrapie-affected sheep or mice than from normal animals and that material from the twenty-third pass of one such cell line was infective for mice. Only two of seven mice reacted, however, so the titre was apparently low and it was not clear whether there had been actual multiplication of the agent or whether this activity was derived from the original tissue.

At Compton we have established a cell line^{2,3} from the brain of a mouse which was showing clinical signs following intracerebral inoculation of the strain of the scrapie agent adapted to mice by Chandler⁴. This line has now been taken through 100 serial passes. To determine whether the agent was multiplying in these cells or merely persisting from the original brain tissue a sample of every fifth passage was titrated intracerebrally in mice. The activity was found to be $10^{2.2}$ ID₅₀/0.05 ml. by the fifth pass and to vary between $10^{1.1}$ and $10^{3.5}$ up to the hundredth.

At the twenty-fifth and again at the sixty-fourth passage level, titrations were made of the level of activity in the cultures at various times after seeding. From the results it seemed that the increase in activity was proportional to the cell count and that this reached a peak at about the time the monolayers were complete. Thereafter the activity seemed to remain constant at least up to 35 days.

No differences were demonstrable when titrations were made in such a way that the cells were kept as intact as possible or when the cells were fragmented by trituration and freezing before the serial dilutions were prepared. It thus seemed that there might be only one active site in each affected cell.

Although the titres which were obtained were comparatively low, the activity in cultures was apparently confined to the cells or cell fragments and the agent was not being released from the cells into the fluid. The proportion of cells to fluid in cultures is of course far lower than that in brain, so that, if the activity is related to cell content, the relative value may not actually be greatly different from that in brain. In cell cultures the scrapie agent seems to multiply in synchrony with and as an integral part of the cells and could be likened to such entities as prophage, plasmids or the transforming factor of an inactivated virus such as SV40, rather than to complete virions which multiply independently. It differs from these entities in its remarkable resistance to various physical and chemical treatments. If the scrapie agent multiplies only in relation to the cells, this could account for the slow, progressive nature of the disease.

Which cells are involved in the multiplication of the transmissible agent of scrapie? Eklund, Kennedy and Hadlow⁵ were impressed by the early development of agent in organs such as the spleen and lymph nodes and suggested that cells of the lymphocytic series might be involved, which might explain the apparent lack of antibody response. But antibody response to antigens such as sheep red cells is apparently unimpaired in scrapie-affected animals⁶ and no morphological changes in the lymphocytes have been observed. Gustafson and Kanitz¹ suggested that astrocytes were the cells observed in their cultures; but although the astrocytes are hypertrophied in scrapie they do not appear to increase in number.

We believe the "target cells" in scrapie may be reticulo-endothelial in origin. Reticulo-endothelial cells are represented in the central nervous system by microglia, which increase in number in scrapie. Although it is not possible to identify the cells in our infected cultures, they resemble morphologically the cells described by Gustafson and Kanitz as astrocytes. When the cells in our cultures were injected intracerebrally into mice, colonies developed in which the histological appearance of the cells suggested that they were mesodermal in origin and more likely to have been derived from microglia than from astrocytes. Support for the belief that cells of the reticulo-endothelial system are concerned in the scrapie reaction is the finding of Haig and Pattison⁷ that capillary cells are active in organ cultures of scrapie brain and that of Buening⁸ that the

cells cultured from scrapie mouse brains were apparently oligodendroglia, microglia and capillary pericytes.

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Taurine—a Possible Neurotransmitter?

APART from the dicarboxylic acids, taurine is the most abundant amino-acid in the nervous system¹, and yet very little is known about its function². It has been suggested that taurine acts as a transmitter; Curtis and Watkins³ have evidence that among other amino-acids, taurine when applied iontophoretically to the vicinity of a neurone in the central nervous system exerts an inhibitory effect on the rate of firing and, furthermore, there is a structural resemblance between γ -aminobutyric acid (GABA) and taurine. There are also similarities in the distribution of both these amino-acids and in the localization of their synthesizing enzymes in different regions of the brain⁴. In addition, after density gradient centrifugation of rat brain homogenate, both cysteinesulphinate decarboxylase activity and taurine has been found to be enriched in the nerve ending fractions⁴. Glutamate and cysteinesulphinate decarboxylases are closely similar enzymes⁵ and their activities increase in parallel during development of rat brain⁴.

In extending this work we have examined other criteria supporting the view that taurine is a transmitter substance. Since GABA and other transmitters are released on stimulation we have first studied the release of taurine on applying

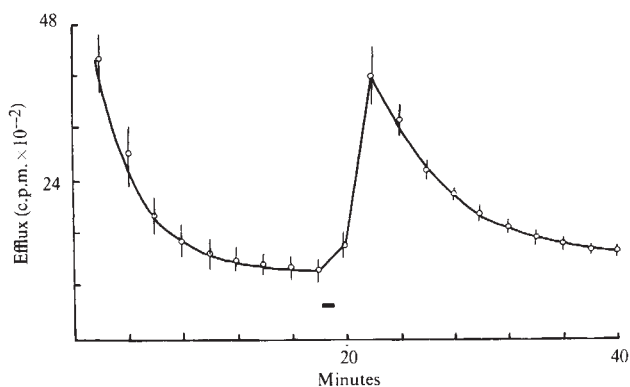


Fig. 1 Release of taurine from stimulated rat cortical slices. Slices preloaded with ³⁵S taurine were incubated in oxygenated Krebs-Ringer medium. ³⁵S-released into the medium is plotted (○) together with the s.e.m. for five estimations. The preparation was electrically stimulated for 1 min (solid bar) and samples of the medium collected every 2 min for estimations of radioactivity.

electrical pulses to tissue slices perfused with Krebs-Ringer medium as described by Srinivasan, Neal and Mitchell⁶. Slices (120 mg) prepared from the cortex of young adult rats (about 150–200 g body weight) were pre-incubated with 1.6×10^{-5} M ^{35}S -taurine for 15 min in 20 ml. of oxygenated Krebs-Ringer medium together with amino-oxyacetic acid (10^{-5} M). (The latter compound inhibits the metabolism of GABA and was included to keep conditions identical to those of the GABA experiments, although it has no known effect on taurine metabolism.) After 30 min the slices were collected by rapid filtration and transferred to a perfusion cell through which taurine-free oxygenated Krebs-Ringer medium was pumped. Samples were collected for radioactivity determination before and after stimulation by rectangular pulses of 60 Hz (duration 2 ms; current 1 mA; voltage 40–80 V). Fig. 1 shows that there is a rapid efflux of ^{35}S from the tissue comparable with that found in similar conditions for GABA⁶. To show the release of ^{35}S to be taurine, brain slices and medium, after and before stimulation, were extracted with 10% (w/v) trichloroacetic acid and washed four times with ether. Extracts were separated on ascending paper chromatography in butanol: acetic acid: water (12:3:5 by volume) and by high voltage electrophoresis in formic-acetic acid buffer, pH 1.86, at 7 kV. In all cases a single peak of radioactivity coincided with that of a standard sample of taurine.

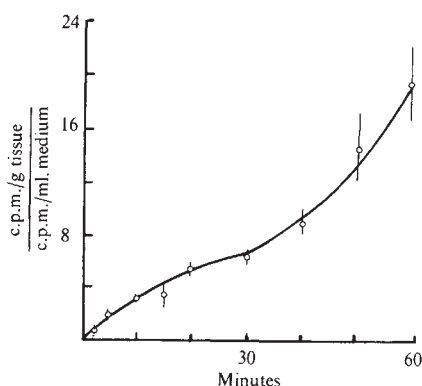


Fig. 2 Uptake of ^{35}S -taurine into rat cortical slices. Brain slices (10 mg wet weight of cortex) were incubated in 10 ml. of oxygenated Krebs-phosphate medium at 37°C . After 15 min ^{35}S -taurine was added to the medium to a final concentration of 5×10^{-7} M. Slices were collected by filtration and rinsed before determining radioactivity at various times of incubation with taurine. Estimations (○) are recorded together with s.e.m. for at least four determinations at each point.

If taurine is released on stimulation, as our experiments suggest, an efficient process for the termination of its action should be available. Since the enzymatic conversion of taurine to isethionic acid in rat brain is too slow to fulfil this role⁷ we have looked for a transport system that could efficiently remove taurine from the extracellular fluid of the brain. In preliminary experiments uptake of very low concentrations of ^{35}S -taurine into slices of rat brain cortex has been found with no detectable metabolism of taurine. Fig. 2 shows the uptake of taurine at 5×10^{-7} M into brain slices; the curve has been fitted to the mean result at each concentration. Further work will be necessary to establish the detailed kinetics of the process and the exact form of the uptake curve. These experiments indicate that uptake is a possible mechanism for the inactivation of taurine and the provisional calculated K_a supports the view. The K_a (apparent K_m) for this uptake process was estimated by measuring the uptake of taurine between concentrations of 2.5×10^{-6} M and 10^{-3} M in the medium into brain slices. The amount of radioactivity in the tissue after 10 min of incubation was used to calculate the rate

of uptake and a value of $K_a = 5 \times 10^{-5}$ M was obtained (compared with GABA where $K_a = 2.2 \times 10^{-5}$ M). Such a process has also been considered likely to apply at synapses where GABA is thought to be released⁸.

It would be premature to conclude that taurine is a transmitter or modulator in the nervous system, for much work on specific antagonists and its localization within identifiable neurones is still essential. Our work does indicate an interesting similarity in release and uptake mechanisms between GABA and taurine, and there are sufficient resemblances in localization and biosynthesis of the two amino-acids to warrant further study.

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DDT in California Sea Lions

WE wish to report extraordinary concentrations of DDT residues* in California sea lions, *Zalophus californianus*, which inhabit year round the coastal waters of California and Baja California, Mexico¹. These waters receive agricultural runoff from California valleys where DDT has been used extensively^{2–4}, and where residues have been increasing in the primary stages of some coastal pelagic food chains⁵.

Tissue samples of blubber, and in some cases, brain liver and muscle, were collected from animals of both sexes and various ages between September 19 and December 10, 1970. These were four apparently healthy adults (one male and three females) shot at San Miguel Island, 56 km from Santa Barbara, and one adult male killed near Año Nuevo Island, 31 km north of Santa Cruz, California, six dying males stranded on beaches between Santa Cruz and San Francisco which were killed in the laboratory, and fourteen fresh male carcasses found on or near Año Nuevo Island. Tissue samples weighed approximately 50 g, were frozen immediately after collection, and stored at -68°C until chromatography was performed according to the methods of Stanley and LeFavoure⁶ and Kadoum⁷.

DDT residues, while present in all samples, predominated in blubber (Table 1). The breakdown of total DDT residues

* DDT residues refer to all the constituents of commercial DDT and its metabolites: *p*, *p'*-DDT (1, 1, 1-trichloro-2, 2-bis(*p*-chlorophenyl) ethane), *p*, *p'*-DDD (1, 1-dichloro-2, 2-bis (*p*-chlorophenyl) ethane), and *p*, *p'*-DDE (1, 1-dichloro-2, 2-bis (*p*-chlorophenyl) ethylene).

was similar for all animals in both blubber and brain (Table 2). An analysis of DDT residues according to sex and age revealed no significant differences.

Table 1 DDT Residues in Healthy, Sick, and Dead California Sea Lions: Means and Standard Deviations, Number of Specimens in Each Group (Parentheses) and Ranges

Group	Total DDT residues			
	p.p.m. wet weight Blubber	p.p.m. lipid weight Brain	p.p.m. lipid weight Blubber	p.p.m. lipid weight Brain
Healthy— killed	906 ± 640	8.6 ± 5.8	1,220 ± 860	85 ± 53
	(5)	(4)	(5)	(4)
	41 to 1,929	0.2 to 16	47 to 2,436	3.1 to 146
Sick—killed	689 ± 164	13 ± 2	1,022 ± 336	140 ± 29
	(6)	(5)	(6)	(5)
	400 to 903	8.9 to 16	552 to 1,387	98 to 190
Fresh carcasses	1,006 ± 645	14 ± 11	1,687 ± 1,280	173 ± 147
	(14)	(6)	(14)	(6)
	258 to 2,678	2.6 to 34	333 to 5,077	23 to 431
Totals: all specimens	911 ± 582	12 ± 8	1,452 ± 1,104	138 ± 105
	(25)	(15)	(25)	(15)
	41 to 2,678	0.2 to 34	47 to 5,077	3.1 to 431

To investigate whether some of the high concentrations in carcasses were due to tissue decomposition after death, blubber and brain samples were collected from a sick animal immediately after death, 2 days later, and once again 5 days after death. During these 5 days, the carcass was left outdoors and covered with burlap sacks. Total DDT lipid values in serial order were 1,267, 1,167 and 1,268 p.p.m. for blubber and 146, 147 and 147 p.p.m. for brain. Evidently, concentrations in these tissues did not fluctuate significantly during this time period.

Table 2 Percentage of DDT and its Metabolites in Blubber and Brain: Means and Standard Deviations

	p.p.m. lipid weight	
	Blubber (N=26)	Brain (N=15)
DDE	93.9 ± 1.9	94.1 ± 3.4
DDD	3.9 ± 1.3	3.3 ± 1.1
DDT	2.2 ± 1.1	2.6 ± 1.2

DDT residues in the muscle of four healthy adults averaged 1.2 ± 0.8 p.p.m. wet weight and 418 ± 246 p.p.m. lipid weight. Mean concentrations in the liver of four healthy adults and three subadults which had been killed in the laboratory, combined, were 17 ± 10 p.p.m. wet weight and 677 ± 229 p.p.m. lipid weight.

The DDT residues in the blubber and brains of these sea lions are higher than those reported in other marine mammals⁸⁻¹³. Maximal values equal those in brown pelicans, *Pelecanus occidentalis*¹⁴⁻¹⁸. We do not know the effects of high DDT concentrations on California sea lions, but studies

on other animals suggest several possibilities—reproductive failure¹⁹⁻²¹, decreased resistance to stressful infectious agents^{22,23}, and an acute toxic effect on the central nervous system²⁴.

Abortions in California sea lions have increased since 1968 on several rookeries off the coast of California and Baja California, Mexico²¹. A systematic census on San Nicolas Island yielded a count of 135 aborted fetuses in 1969 and 442 in 1970, more than a three-fold increase from one year to the next²⁵. We counted 283 one-week-old pups at Islas San Benito and Isla San Martín, Mexico, between May 25 and May 28, 1971—133, or 47%, of them were dead.

Between August 25 and November 30, 1970, Mrs J. Schonewald of the California Academy of Sciences received 420 reports of sick or dead sea lions observed between Monterey and Fort Bragg, California. An epidemic was publicized nationally (for example, *San Francisco Chronicle*, October 14, 1970, and *New York Times*, October 15, 1970) with death being attributed to the bacterium, *Leptospirae*, which was isolated in some of the animals.

It is doubtful that the increased mortality resulted from an acute toxic effect of DDT since concentrations in carcasses were not significantly higher than in the living animals. Second, concentrations of DDD and DDT, combined, in the brains of carcasses are far below the lower limit of 30 p.p.m. suggested as the direct cause of death in birds²⁴.

The potential danger of organochlorine pesticides to pinnipeds warrants our concern, for it involves not only these marine mammals but man, whose diet often includes fish and cephalopods.

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Light Flashes produced in the Human Eye by Extremely Relativistic Muons

It is generally agreed that the light flashes observed by astronauts on Apollo missions 11-14¹ were produced by the passage of heavy cosmic ray nuclei through the eye. The exact mechanism has been a subject of some controversy, however, most workers leaning towards either Čerenkov radiation²⁻⁴ or direct ionization and excitation of molecules at the retina^{1,5,6}. Light flashes similar to the Apollo observations have been reported to result from irradiations of the eyes by X-rays^{5,7}, neutrons^{5,6,9,10} and slow alpha particles⁸, all of which are more likely to involve ionization and excitation than Čerenkov radiation. Early indications that the human eye might be sensitive to individual relativistic singly charged particles¹¹ have not been borne out by calculations³ or by recent observations⁵.

This report describes a test of the ability of the human eye to detect Čerenkov radiation when it is generated with sufficient intensity by the passage of a burst of relativistic charged particles through the vitreous humour and retina of the eye. The small values of LET and total dose involved in producing the bright extended flashes described below seem to rule out any significant contribution from direct ionization and excitation. All observations were made during two short periods of exposure to a beam of 6 ± 2 GeV/c muons at the Brookhaven AGS. Red X-ray dark adapting goggles were worn for at least 0.5 h before exposure and observations were made in a completely darkened state under improvised cloth and leather hoods with the muons entering the eye through the side of the head. The muons arrived spread over pulses of 440 ms duration with a time interval of 2 s between pulses. The flux density of muons in each pulse was approximately 3×10^3 muons cm⁻².

The phenomena appeared as bright diffused flashes which appeared and disappeared with the beam pulses and seemed to fill the periphery of the field of view but never the entire field of view. When I turned away from the beam so that it entered from the rear of the head, the flashes were no longer clearly distinguishable. The coincidences between announced flashes and the beam pulses were checked as well as my ability to tell when my eyes were centred in the active beam.

Localized areas of the retina function as units at threshold levels of signal. The areas of these summation units range

from 10^{-6} cm² at the fovea to about 9×10^{-4} cm² on the periphery with an average of roughly 8×10^{-5} cm². Signals generated in a unit within an interval of 0.1 s are effectively in coincidence. The number of such coincidences required to induce a visual sensation is somewhere between two and twenty, ten being typical of most measurements¹².

There is some possibility that the flashes observed in this experiment are similar in origin to the haze reported in X-ray^{5,7} and neutron⁶ irradiations of the eye, phenomena that would certainly not involve Čerenkov radiation. But the flux rate that resulted in bright extended flashes corresponds to the passage of three muons per pulse through a 9×10^{-4} cm² summation unit on the periphery or less than 1 in each 0.1 s interval of beam. This rate of dose, 0.026 mr in 0.1 s, is a factor of ten smaller than the X-ray doses over the same or shorter intervals that were required for even a slight haze^{5,7} despite the similarly low LET associated with both radiations. Moreover, the 10 s persistence reported for the neutron produced haze⁶ was not observed in this experiment.

On the other hand, the number of "visible"³ Čerenkov photons in each 0.1 s of pulse that can be expected to accompany the muons through the retinal summation units has been calculated¹³ as 40 on the periphery, 0.1 at the fovea and about 4 for a unit of average area. If we assume the probability of absorbing each photon incident on the periphery to be 20%, the value measured at 10° - 20° eccentricity^{14,15}, then a threshold value of ten absorbed photons should be exceeded in the muon experiment for 26% of the peripheral units in each 0.1 s interval of the 0.44 s pulse and essentially nowhere else on the retina. Although calculations of this type are necessarily crude as a result of our incomplete knowledge of the peripheral region of the retina, they do indicate Čerenkov radiation as the most plausible mechanism for producing the flashes described above.

I thank members of the Columbia, Harvard, National Accelerator Laboratory, and Rochester University collaboration who designed and operated the muon facility at the Brookhaven AGS for help, Professor L. Lederman for helpful suggestions and Dr Paul Bottino for help with the observations.

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- ¹³ McNulty, P. J., *Document No. AFCRL 71-0377* (Air Force Cambridge Research Laboratories, 1971).
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BOOK REVIEWS

Vertebrate Past

Vertebrate Palaeozoology. By Everett C. Olson. Pp. xiii+839. (Wiley Interscience: New York and London, May 1971.) £14.25.

It is rare—and highly refreshing—to find a textbook in any subject which makes a completely new approach. This Professor Olson has achieved. The conventional textbook tells the reader what is known; where difficulties arise they are glossed over or sometimes ignored altogether. The intention is to produce a self-contained synthesis which gives the student a clear and intellectually satisfying picture of the basic structure—and the excellence of the textbook varies inversely with the number of inconvenient facts omitted.

Vertebrate Palaeozoology has a different aim and follows a different pattern. The author sets out to give an overall picture of the present state of the science, to relate the animal to its environment and to clothe the hard parts in living flesh. But a much more significant and valuable innovation is that we are spared none of the difficulties, rather are they carefully spelled out for us. Thus the man who is teaching in any branch of vertebrate palaeontology can get an up to date picture of the state of knowledge in that field, and, what is perhaps more important, the certainty of that knowledge. The research worker can see how work is progressing in fields related to his own, and can perhaps find it valuable in suggesting research problems for his graduate students. Most valuable of all, however, the student can really see what science—as exemplified by vertebrate palaeontology—is all about—how it consists at any time of a mass of facts: some connected reasonably firmly by theory, some strung together by tentative and rather unsatisfactory hypotheses and some just sticking out like sore thumbs and refusing to fit in comfortably anywhere. As pedagogic methods change and student studies increasingly range more widely and, as an inevitable concomitant, become shallower, the experience of being asked a question by a student, answering honestly “I do not know” and seeing the look of outraged horror on his face, will become commoner. Professor Olson’s book is a useful corrective to students falling into the error that either their teachers or their textbooks embrace, in their own field, the whole of knowledge.

The book is divided into four sections. The first deals with the history of the Vertebrata in broad outline and in a rather summary manner—it is short (52 pages). The second section is entitled “Vertebrate Morphology and Function”; it is larger (114 pages); and its scope is adequately illustrated by its title. The third section is considerably longer than either of the first two (262 pages) and is entitled “The Classification of the Vertebrates”. This covers rather more than is suggested by the title of the section, and includes a considerable amount on the phylogenies of the taxa.

The last section is the longest of all (385 pages) and deals in detail with five special topics: the problems of life in water, the radiation of the Actinopterygii, the conquest of land, the origins of the Mammalia and the influence of palaeogeography on evolution. This section inevitably overlaps to some extent with the one that precedes it. The book is well illustrated but has no plates.

The only major criticism of the book is its price: £14.25 is ridiculous for such a book and ensures that its only purchasers will be libraries and the more affluent senior workers. This is a pity, as it would be invaluable for teaching graduate students and undergraduates taking advanced courses; and at a more realistic price it could well be prescribed as a class textbook. K. A. KERMACK

Travels of Banks

Joseph Banks in Newfoundland and Labrador, 1766. His Diary, Manuscripts and Collections. By A. M. Lysaght. Pp. 512+103 plates. (Faber and Faber: London, August 1971.) £15.

THE name of Joseph Banks is most familiar as the associate of James Cook on his epic voyage around the world in the Endeavour (1768–71), and since the bicentenary celebrations in Britain and Australia this connexion has been many times reinforced. Banks’s part in that voyage has to some extent been misinterpreted by later commentators who have represented him as the gentleman amateur, a dilettante naturalist of wide interests but little application. In other eyes Banks was the organizing genius and employer of a well chosen band of scientists and illustrators whose contribution to natural history would have been profound had not sickness and death broken up his team on the return journey.

That Banks made a voyage to Newfoundland and Labrador in 1766 is little known, and in many ways this cruise on the Niger was a rehearsal for the later Endeavour voyage. There is no doubt that it furthered his interest and competence in natural history and set a standard of biological exploration which was to be maintained throughout his later voyages. In April 1766 Banks and Constantine John Phipps joined the Niger, a 32-gun frigate of 679 tons. They spent the months from May to October in the region between St John’s, Newfoundland, and Labrador, collecting, exploring, and studying the local inhabitants, their life, economy, and particularly their operation of the great fisheries there. In these five months (four in fact as Banks was ill for most of July) he collected or recorded 340 species of plants, 91 species of birds, and a number of fishes and invertebrates. In addition he kept a journal of his general activities as well as more detailed botanical and zoological notebooks. This all amounted to a considerable achievement for a young man of 23 on his first expedition, especially in the cramped and rigorous conditions of a naval frigate of 1766.

Dr Averil Lysaght’s book is a scholarly appreciation of Banks’s work in this region of North America. She has printed his diary (now in the library of the Royal Geographical Society of Australia, South Australian Branch), the detailed descriptions of animals and a few plants (now in McGill University Library), and a catalogue of the names and localities of the plants (now in the British Museum (Natural History)). Strangely, Banks’s journal is a very impersonal affair and the man remains something of an enigma. Nowhere does he allow his feelings to obtrude on the factual statements of the day’s activities, and his stay in Lisbon at the end of the journey sounds to have been a dull and uneventful time which impression is, however, shown to be false by the relevant letters and notes. These major manuscripts are supplemented by lists of the animals preserved in spirits or of birds’ skins, and other original observations, but the whole is welded together with such a wealth of explanatory notes and biographical detail that there is rarely a question comes to mind that is not already answered in a complete manner elsewhere in the book. Lysaght also sheds some interesting new light on Banks’s previous acquaintance with Cook (for it has been claimed that they

first met on the deck of the Endeavour just before she sailed in 1768). In fact it seems that Cook and Banks could well have met in St John's harbour in 1766, for Cook's surveying vessel Grenville arrived there at least a day before the Niger sailed, and it seems impossible that the Niger's passengers would not have seized the opportunity to meet the already respected navigator and cartographer.

The illustrations in the volume deserve special mention. Both the twelve colour plates, those of birds painted by Sydney Parkinson and Peter Paillou, and the plants by G. D. Ehret, and the 91 monochrome illustrations add greatly to the interest of the work. The quality of reproduction of the plates and the presentation of the whole book reflect great credit on the designer and publishers.

This masterpiece of historical scholarship will stand as a fitting memorial to Banks's work, and must be some reward to the author for many years of unstinting study of eighteenth century natural history.

ALWYNE WHEELER

Effects of the Pill

Metabolic Effects of Gonadal Hormones and Contraceptive Steroids. By Hilton A. Salhanick, David M. Kipnis and Raymond L. Vande Wiele. (Based on a Workshop held in Boston, Massachusetts, December 1-5, 1968.) Pp. xxix + 762. (Plenum: New York and London, 1969.) \$27.50.

WITH the introduction of oral contraceptives we have experienced the first, and still only major, application of mass medication to previously healthy and asymptomatic, but potentially diseased, individuals. It was inevitable that such extensive usage of the products of the pharmaceutical industry would bring in its wake a host of drug-induced and drug-exacerbated diseases and that this would in turn lead to intensive investigation into their prevalence and nature.

Sufficient evidence was available by 1968 to justify holding a conference devoted to the metabolic effects of gonadal hormones and contraceptive steroids, the proceedings of which became the subject of this review. Although most biochemical changes produced by the contraceptive steroids are not associated with overt disease the data collected together in this volume leave no room for doubt that as a group this class of compounds has profound metabolic effects on most, if not all, systems of the body. They do not, as was once supposed, act only on the endocrine and reproductive organs.

This book is composed of fifty-two chapters, each written in the form of an "original" article, which are grouped to-

gether into nine separate sections, each dealing with a different body system. The editors have wisely not followed the current fashion of including verbatim all of the discussion after each paper. Instead they have selected and pruned it. This enhances rather than detracts from the value of the book which is a commendable record of the experimental basis of knowledge of the metabolic and biochemical effects of the steroidal contraceptive agents up to and including 1968.

VINCENT MARKS

Proteinase Action

Proceedings of the International Research Conference on Proteinase Inhibitors, Munich, November 1970. Edited by H. Fritz and H. Tschesche. Pp. ix + 304. (Walter de Gruyter: Berlin and New York, 1971.) 108 DM.

THIS volume contains the proceedings of the first international research conference on proteinase inhibitors which was held in Munich in November 1970.

The conference covered a wide area and started quite rightly with four contributions on proteinase inhibitors in human medicine. Following these contributions, which provide a basic orientation, there are three papers on specific isolation methods. Then both the theory and methods of studying the inhibition mechanism are outlined by six contributors. The conference then passed further afield and the book contains articles on inhibitors from plant tissues. The most well understood proteinase inhibitor, the basic bovine compound, is thoroughly chewed over by eight different authors. In this section I found much of the meat of the proceedings. Inhibitors from pancreas, seminal fluid and miscellaneous sources are also described. The subject is certainly comprehensively covered.

Heimburger's group from the Behringwerke, Marburg, describe the isolation of six different proteinase inhibitors from human serum, all of which contain some carbohydrate. Although an earlier hypothesis that α_2 macroglobulin had an insulin carrier function was not confirmed, it was observed at the conference that this α_2 macroglobulin is identical to the single protein of serum which can bind zinc. It has been reported to be high in diabetes.

The isolation methods described include water insoluble trypsin resins (Fritz *et al.*, who were the first to use affinity chromatography in this area), chymotrypsin-sepharose (Feinstein) and trypsin-sepharose (Kassell and Marcinişyn).

The molecular structures of some crystalline proteinases are outlined by Shotton who shows the common structural pattern and their specific binding sites. To this is added the very satis-

fying and elegant results of Huber and co-workers in Munich who have established the atomic structure of the basic trypsin inhibitor of bovine organs. This molecule has several very interesting structural features; it is dominated by a substantial amount of anti-parallel β -pleated sheet and also contains some polyproline II helix.

Many of the contributions show clearly that this subject is becoming extremely well defined as the techniques of protein chemistry are increasingly applied to the problem. This work will be of great value to everybody interested in proteinase action and its control.

S. SHALL

Mass Spectrometry

Mass Spectrometry. Vol. 1. Senior Reporter D. H. Williams. Pp. ix + 323. (The Chemical Society: London, February 1971.) £7.

THIS is the first of a series of specialist volumes promised and it is appropriate to ask what its intended function might be. It seems to me to represent an extension of current Chemical Society publications, in fact to be a mixture of two of them. The first, sixth and seventh chapters are reminiscent of *Quarterly Reviews* and the remainder of *Annual Reports*. A clearer sense of purpose may emerge in later volumes.

The rapid expansion of mass spectrometry is well indicated by the contents of this book which is necessarily very comprehensive, not to say compressed. In particular there are chapters on alternative methods of ionization and analysis; computerized data acquisition and handling; gas chromatography-mass spectrometry; energetics, kinetics and ion structures; reactions of specific functional groups; natural products including oligosaccharides; and organo-metallic and coordination compounds. This last chapter clearly indicates the rapid rise in the popularity of metallo-cene chemistry for, notwithstanding the length of this section, there is barely occasion for more than a mention of all the results reported.

There are voluminous references and an adequate index. The chapters are all written by well known practitioners in the respective fields and are, in general, clearly written and readily understood. The book is well produced and although rather expensive is not excessively so for these days.

The spelling is sometimes unusual, for example colinear (page 23 legend Fig. 3) and there are some trivial errors (page 67, line 17 should read "mass spectrometer"). Moreover, SI units are nowhere mentioned, the traditional Torr and kilocalories being preferred.

ROWLAND I. REED

CORRESPONDENCE

Synthetic Pyrethrins

SIR,—We would like, on behalf of the pyrethrum industry, to comment on the article "Pyrethrin Prospects" (*Nature*, 233, 441; 1971).

We cannot agree with your viewpoint that the pyrethrum industry will suffer a "fate" similar to certain natural products. Incidentally, the one you mention, quinine, is said to be doing extremely well.

It may be worthwhile to look at some facts about synthetic pyrethroids. Initially, Allethrin, which got off the ground in America as a strategic contingency measure, declined at the end of the war and survived in Japan only under a heavy discriminatory tariff against imported natural pyrethrum. This tariff being still in existence, it comes as no surprise that the new generation of synthetics being manufactured there are enjoying similar protection. To put the new synthetic products in a nutshell, what you get, at least for aerosols, is either an economic level which results in good kill with medium knockdown, or an uneconomic level which gives good knockdown and overkill: hardly an easy choice for the formulator.

The point about lower acute toxicity rates (rat) for synthetics is interesting academically, though it does not summarily relegate natural pyrethrins into a separate toxicity class. At least one synthetic pyrethroid sank without trace during its development stage, because of high mammalian toxicity. We do not know if the toxicity figures for synthetic products represent chemically pure specimens or the practical commercial materials, but believe that the manufacturers ought to state what solvents, aromatic or otherwise, are used. Again, no assay method exists for determining synthetics in the presence of aromatic solvents, pyrethrins and synergists.

We are rather surprised that natural pyrethrins should be labelled as causing sneezing, when for years aerosol formulators have known¹ that one or two commercially produced synergists are more indictable. Indeed, lately one of the new synergists, "Tropital", has appeared on the market claiming specifically "non-sneezing" properties. We cannot agree that the prices which you quote for synthetics make them competitive with pyrethrins. All pyrethroids are certainly out of the class of organochlorides, and

always have been, but this is a point hardly at issue these days. The new technique of ultra-low volume dispersment (ULV), especially by aircraft, opens up a huge range of uses for pyrethrins hitherto closed, due to the adverse economics of the quantities involved. In addition, interesting new repellency factors are being established about pyrethrum, which also offer new uses.

Where the synthetics have succeeded has been due to recent shortfalls in the production of natural pyrethrum. This was caused in East Africa by a switch-over to high content pyrethrum clones, which coincided with unusual drought conditions and led to the loss of many plants and a severe overall setback in production. The rationale of producing high content pyrethrum plants, however, is that, with no more effort, two or three times the quantity of pyrethrum can be produced from the same weight of dried flowers, and the economics of this are obvious.

Yours faithfully,

D. R. MACIVER
Director

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PO Box 420,
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¹ Zucker, A., "Investigation of Purified Pyrethrum Extracts", *Annals of Allergy*, 23 (1965) (vide *Pyrethrum Post*, 8 (3), 7-9).

Phosphate Detergents

SIR,—I think that the article by your Washington correspondent putting down the environmentalists ("Algae Better Than Burns", *Nature*, 233, 229; 1971) was in itself somewhat hasty. The movement towards phasing out phosphates certainly did not occur overnight. And I find it hard to believe that the detergent manufacturers have really spent "millions of dollars" testing alternatives. I believe that he has simply quoted company rhetoric without verifying it, which is as reprehensible in an editorial as it would be in a scientific paper. After all, what is needed to perform a scientific test of a detergent's effectiveness but a washing machine and a few dirt-stained collars? This used to be proved nightly on television. The abuses to the environment (and thus to ourselves) can and will be

stopped: the ecology movement is not going to go away. Once that is accepted, the priorities become clear. To have made a wrong move (if indeed it was) on the way to the right goal is not a tragedy. I think that the reason so many people have been jumping into ecology with both feet lately is that they have discovered the indescribable bliss that comes from ceasing to do something wrong.

Yours faithfully,

MARK WIRTH

*10212 Capitol View Avenue,
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SIR,—In three recent issues of *Nature* (233, 229, 295, 362; 1971), you have discussed the dilemma over phosphates in detergents. But there is one question with which you have not concerned yourself: are phosphates necessary to get a wash clean? This, I believe, is a crucial question. Detergent manufacturers have claimed that everyone's wash will come out a tattletale-grey if we stop using detergents, whether these detergents contain phosphates or a phosphate-substitute. This is simply not true.

I tried an experiment where I put two extremely dirty laboratory coats in with my regular weekly laundry. I used only soap and washing soda. Both coats came out white. I will not deny that phosphates do help to get a wash clean, but I am convinced that a high percentage (40-60 per cent) is quite unnecessary. (Another point: high-phosphate washday products are invariably high priced.)

For these reasons I cannot go along with the Environmental Protection Agency's unreserved endorsement of phosphates. If they had endorsed low-phosphate (10-20 per cent) detergents only, I could understand that. But to say in effect that high-phosphate detergents are necessary for a clean wash, and that we have no choice but to put up with the pollution from them, is economic and ecologic nonsense.

Yours faithfully,

GRETCHEN LUEPKE

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Errata

In the article "Unique Bedrock and Soils associated with the Teesdale Flora" by G. A. L. Johnson, D. Robinson and M. Hornung (*Nature*, **232**, 453; 1971), an unfortunate transposition of lines occurred. The first three lines of column 2, page 454, should follow immediately after the fifth line of the section "Whin Sill and Metamorphism" in the first column of the same page.

In the article "Extended Sleep and Performance" by John M. Taub, Gordon G. Globus, Eric Phoebus and Robert Drury (*Nature*, **233**, 142; 1971), the first sentence of paragraph 4 should read: "Table 1 shows the average number of minutes spent in each sleep stage, wakefulness, and total sleep time during both nights of each sleep condition". It should be noted that Table 1 does not include total time in bed, and that reference 8 is not cited in the text.

In the article "Retraction and Expansion of the Dendritic Tree of Motor Neurones of Adult Rats induced *in vivo*" by B. E. H. Sumner and W. E. Watson (*Nature*, **233**, 273; 1971), the second sentence of paragraph 2 should read: "200 μ m low viscosity nitrocellulose . . .".

Reports and Publications

not included in the monthly Books Supplement

Great Britain and Ireland

The Natural Rubber Producers' Research Association. Thirty-third Annual Report. Pp. 48. (London: The Natural Rubber Producers' Research Association, 1971.) [197]
King Edward's Hospital Fund for London Annual Report for 1970. Pp. 39. (London: King Edward's Hospital Fund for London, 1971.) [197]
Glasshouse Crops Research Institute. Annual Report for 1970. Pp. 210. (Rustington, Littlehampton: Glasshouse Crops Research Institute, 1971.) £1.25. [197]
Advisory Committee on Gas Council Researches—Report 1969-70. Pp. 11. (Leeds: The University, 1971.) [197]
Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 269, No. 1201 (15 July 1971): A Unified Theory of Conjugate Flows. By T. B. Benjamin. Pp. 587-

647. £1.50; \$3.90. Vol. 270, No. 1202 (16 July 1971): A Discussion on Solar Studies with Special Reference to Space Observations. Arranged by the National Committee on Space Research under the leadership of Sir Harrie Massey, C. W. Allen, A. H. Gabriel, B. E. J. Pagel and R. Wilson. Pp. 1-195 + plates 1-10. £5.90; \$15.40. (London: The Royal Society, 1971.) [197]
Proceedings of the First International Symposium on Biological Control of Weeds, March 1969. Edited by Dr. F. J. Simmonds. (Miscellaneous Publication No. 1 of the Commonwealth Institute of Biological Control, Trinidad.) Pp. vii+110. (Farnham Royal, Slough: Commonwealth Agricultural Bureaux, 1970.) £2. [197]
Department of Education and Science. Scientific Research in British Universities and Colleges, 1970-71. Vol. 1: Physical Sciences. Pp. xxiii+841. £6.50 net. Vol. 2: Biological Sciences. Pp. xxiii+706. £6 net. Vol. 3: Social Sciences (Including Government Departments and Other Institutions). Pp. xxxii+608. £5.50 net. (London: HMSO, 1971.) [207]

Africa, America, Asia, Europe, Oceania: Family Planning in Five Continents. Pp. 34. (London: International Planned Parenthood Federation, 1971.) [207]
The Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom. A Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom in 1972-73, excluding Degree Courses for External Students. Pp. 288. (London: The Association of Commonwealth Universities, 1971.) £1.30. [227]
International Index of Laboratory Animals. Second edition. Pp. viii+89. (Carshalton, Surrey: Medical Research Council, Laboratory Animals Centre, 1971.) £1. [227]
The Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 4, No. 1: The Stratigraphy and Structure of the Moinean and Dalradian Metasediments of the Mullet Peninsula, Co. Mayo, Ireland. By J. S. Sutton. Pp. 1-14. 50p. Vol. 4, No. 2: Some Conspicuous Participants in Palaeozoic Symbiosis. By C. H. Holland. Pp. 15-26+plate 2. 50p. Vol. 4, No. 3: The Lower Carboniferous (Dinantian) Stratigraphy of the Carrigaline Area, Co. Cork, Ireland. By Michael E. R. Le Morvan. Pp. 27-36+plate 3. 50p. Vol. 4, No. 4: A Collection of Irish Bank Vole Skulls. By J. S. Fairley. Pp. 37-44. 50p. Vol. 4, No. 5: Preliminary Palaeomagnetic Results from Some Irish Limestones of Carboniferous Age. By I. R. McAulay and P. Morris. Pp. 45-55. 40p. (Dublin: Royal Dublin Society, 1971.) [227]
The Countryside Commission. Countryside Information Directory. (London: The Countryside Commission, 1971.) [227]

Current Contents—Science and Technology, Vol. 1, No. 1, January 1971. Pp. 1-94. Published monthly. Subscription: Rs. 36; £4.15; \$10. (Gurgaon, Haryana, India: Indian Documentation Service, 1971.) [137]
Atlantide Report No. 11: Scientific Results of the Danish Expedition to the Coasts of Tropical West Africa, 1945-1946. Pp. 243. (Copenhagen: Danish Science Press, Ltd., 1970.) 90 Danish Kr. [137]
US Department of the Interior: Geological Survey. Professional Paper 521-C: Geology of the Paleozoic Rocks, Navajo and Hopi Indian Reservations, Arizona, New Mexico and Utah. By E. H. Irwin, P. R. Stevens and M. E. Cooley. Pp. iii+32+2 plates. Professional Paper 521-D: Hydrogeology of the Cenozoic Igneous Rocks, Navajo and Hopi Indian Reservations, Arizona, New Mexico and Utah. By J. P. Akers, J. C. Shorty and P. R. Stevens. Pp. v+18+2 plates. Professional Paper 521-E: Mesozoic Stratigraphy of the Santa Rita Mountains, Southeast of Tucson, Arizona. By Harald Drewes. Pp. v+81. \$1.50. (Washington, DC: Government Printing Office, 1971.) [137]
New Zealand Department of Scientific and Industrial Research: Geophysics Division. New Zealand Seismological Report—Inangahua Earthquakes 1968. By R. D. Adams, M. A. Lowry and D. E. Ware. Pp. 280+12 maps. (Wellington: Government Printer, 1971.) [157]
Metropolitan Life. 1970 Report to Policyholders. Pp. 16. (New York: Metropolitan Life Insurance Company, 1971.) [157]
Background Study for the Science Council of Canada. Special Study No. 15: Scientific Activities in Fisheries and Wildlife Resources. By D. H. Pimlott, C. J. Kerswill and J. R. Bider. Pp. 191. (Ottawa: Information Canada, 1971.) \$3.50. [197]
Australian Journal of Zoology, Supplementary Series. Supplement No. 4: The Tabanidae (Diptera) of Australia. V. Subfamily Tabaninae, Tribe Tabanini. By I. M. Mackerras. Pp. 54. (East Melbourne: CSIRO, 1971.) [197]
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